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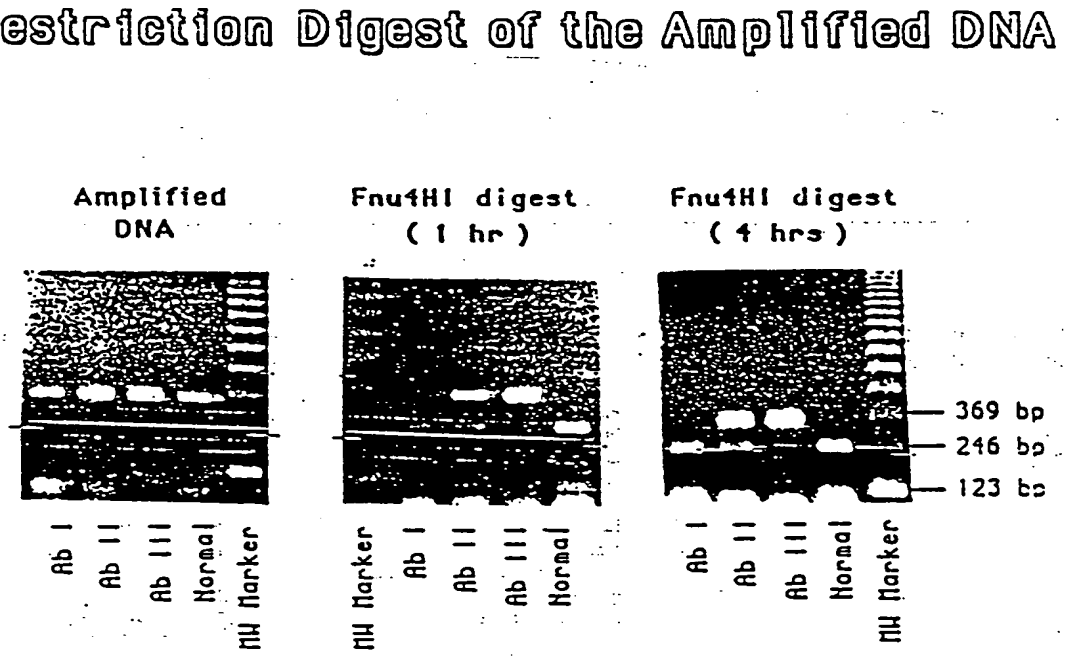

(57) Abstract

Methods for detecting the presence of selected mutations, such as the Thr-601 mutation and the Phe-355 mutation, in the plasminogen of a patient are disclosed. The methods include exposing amplified genomic DNA to a restriction endonuclease capable of differentially cleaving mutant and wild-type plasminogen DNA sequences, and analyzing the exposed DNA to detect the presence or absence of cleavage fragments diagnostic for the selected mutation. Diagnostic kits for the rapid detection of the selected mutation are also disclosed.

* (Referred to in PCT Gazette No. 04/1990, Section II)

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LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirement of unity of invention and relates to several inventions or groups of inventions,

namely:

1. Claims 1-3,5-7, and 4,8-13(partially): A method for detecting deletions at a plurality of DNA sequences; the use of this method for detecting muscular dystrophy, primers therefor and DNA sequences identical or complementary to these primers.

2. Claim 4(partially): The use of the method of the first subject for detecting transcarbamylase deficiency.

3. Claim 4 (partially: The use of the method of the first subject for detecting hypoxanthine phosphorybosyltransferase deficiency.

4. Claim 4(partially): The use of the method of the first subject for detecting steroid sulphatase deficiency.

5. Claim 8(partially): The method of the first subject wherein a pair of primers which amplifies a DNA sequence of the human beta-globin gene is used (pair 8).

6. Claim 8 (partially): The method of the first subject wherein 2 pairs of primers which amplify a DNA fragment linked to the alpha-1-antitrypsin deficiency are used (pairs 9 and 10).

7. Claims 14-17 and 9-13(partially): DNA sequences not falling under the cope of the first subject.

The above analysis is based on a partial search and on anticipation of the common inventive concept as present in the first claim.

As for the hitherto unsearched potentially inventive concepts of the subjects 2-7 the further search could reveal that they are already part of the state of the art and therefore no acknowledgement of unity of invention of the above identified subjects 2-7 is implied by the indication of topics listed above.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 89/02731

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC : 4 C 12 Q 1/68, // C 07 H 21/04

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System [8]	Classification Symbols
IPC 4	C 12 Q

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	Proc. Natl. Acad. Sci. USA, volume 85, January 1988, D.R. Engelke et al.: "Direct sequencing of enzymatically amplified human genomic DNA", pages 544-548 see the whole article	1-4,18,19
A	EP, A, 0258017 (CETUS CORP.) 2 March 1988 see abstract; page 13, line 54 - page 21, line 39	1-4,18,19
A	EP, A, 0237362 (CETUS CORP.) 16 September 1987 see the whole document	1-4,18,19
A	EP, A, 0256630 (HOWARD HUGHES MEDICAL INSITUTE) 24 February 1988 see the whole document	1-4,18,19
A	WO, A, 84/01389 (MASSACHUSETTS INSTITUTE OF TECHNOLOGY) 12 April 1984	1-4,18,19

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* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
12th October 1989	20 NOV 1989
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	T.K. WILLIS

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
	see abstract; claims 1-12	
A	FEBS Letters, volume 213, no. 2, March 1987, Elsevier Science Publishers B.V. (Biomedical Division), M. Forsgren et al.: "Molecular cloning and characterization of a full-length cDNA clone for human plasminogen", pages 254-260 cited in the application	
A	Proc. Natl. Acad. Sci. USA, volume 79, October 1982, T. Miyata et al.: "Plasminogen Tochigi: inactive plasmin resulting from replacement of alanine-600 by threonine in the active site", pages 6132-6136 cited in the application	

INTERNATIONAL SEARCH REPORT

Int tional application No.
PCT/US94/0 308

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ANALYTICAL CHEMISTRY, VOL. 63, No. 5 (01 MARCH 1991) J.V. Sweedler et al"Fluorescence Detection in Capillary Zone Electrophoresis Using a Charge-Coupled Device with Time-Delayed Integration", pp496-502.	

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 8902731 SA 29911

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 15/11/89
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0258017	02-03-88	AU-A- 7729887	19-05-88
		JP-A- 63102677	07-05-88
EP-A- 0237362	16-09-87	AU-A- 6996287	17-09-87
		JP-A- 62214355	21-09-87
EP-A- 0256630	24-02-88	AU-A- 7628187	04-02-88
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		US-A- 4786718	22-11-88

METHOD FOR DETECTING ABNORMAL GENES

Technical Field

The present invention is related generally to the detection of abnormal genes. More specifically, the invention provides methods for detecting the presence of abnormal plasminogen genes, such as a gene encoding Thr-601 plasminogen or a gene encoding Phe-355 plasminogen.

Background of the Invention

In order to understand the mechanisms and genetics of human diseases, it is important to identify DNA and protein markers that indicate the presence of genetic defects in populations and families. For example, deficiencies in protein C, protein S, antithrombin III, heparin co-factor II, tissue-type plasminogen activator and plasminogen have been identified as the cause or at least part of the cause of a predisposition for thrombosis in some patients with hereditary thrombophilia (for review, see Bauer and Rosenberg, Blood 70:343-350, 1987, and Mannucci and Tripodi, Thromb. Haemostas. 57:247-251, 1987).

Plasminogen is a single-chain proenzyme that is converted to an active two-chain form (consisting of an A and a B chain connected by two disulfide bonds), called plasmin, by activators such as tissue-type plasminogen activator, urokinase, and streptokinase. Plasmin digests fibrin clots to form soluble fibrin degradation products. In addition, plasmin is thought to play an important role in various biological reactions, such as inflammation, tissue development and remodeling, processing other molecules, etc.

The primary structure of plasminogen (790 amino acid residues) was established by Sottrup-Jensen et al. (Prog. Chem. Fibrinol. Thrombol. 3:191-209, 1978). This

amino acid sequence has been confirmed by cDNA sequencing (Malinowski et al., Biochemistry 23:4243-4250, 1984 and Forsgren et al., FEBS Lett. 213:254-260, 1987), which indicated the presence of an additional Ile residue at position 65. Accordingly, plasminogen contains 791 amino acids (See Figure 1). The A chain of the molecule consists of the activation peptide (77 amino acid residues) and five disulfide bond-folded structures called "kringles" (about 90 residues each). The B chain contains the activation site (between Arg-561 and Val-562), the active site His-603 residue region, the active site Asp-646 residue region, the region which is linked to the heavy chain by a disulfide bond, the active site Ser-741 residue region, and the C-terminus (amino acid numbers used herein refer to the sequence shown in Figure 1). The first kringle structure (K1) in the A chain of plasminogen is responsible for its binding to fibrin (Thorsen et al., Biochim. Biophys. Acta. 668:377-387, 1981). The B chain of plasminogen carries all three active sites essential for catalytic function as a serine protease.

There are at least several genes in the human genome that are homologous to that of plasminogen, such as apolipoprotein(a) (McLean et al., Nature 330:132-137, 1987). Apolipoprotein(a) contains 37 copies of plasminogen kringle 4 and one copy of plasminogen kringle 5. It also contains a serine protease domain that is highly homologous with the B chain of plasminogen.

Several cases of a molecular abnormality of plasminogen in association with a complication of thrombosis have been reported (Aoki et al., J. Clin. Invest. 61:1186-1195, 1978; Kazama et al., Thromb. Res. 21:517-522, 1981; Wohl et al., Thromb. Haemostas. 48:146-152, 1982; Soria et al., Thromb. Res. 32:229-238, 1982 and Scharrar et al., Thromb. Hemostas. 55:396-401, 1986). These abnormalities have been found most frequently in Japan, but have also been reported in other populations. By an analysis of the plasminogen molecules from these patients, it has been

shown that an amino acid substitution of Thr for Ala-601 in the B chain results in the generation of an inactive plasmin molecule (Sakata and Aoki, J. Biol. Chem. 255:5442-5447, 1980; Miyata et al., Proc. Natl. Acad. Sci. USA 79:6132-6136, 1982; Miyata et al., J. Biochem. 96:227-287, 1984). However, the nature of the underlying abnormality at the DNA level has not heretofore been determined, and other plasminogen disorders have not been characterized.

Since plasminogen is the key enzyme in the fibrinolytic system, responsible for removing fibrin clots from circulation, individuals with abnormal plasminogen or a plasminogen deficiency develop thrombosis. Given the gene frequency of approximately 0.02 among Japanese, the expected number of homozygotes with the Thr-601 plasminogen variant is calculated to be about 50,000 in Japan (population of approximately 125 million). A few homozygotes have been found; however, the homozygous condition is expected to be lethal in most cases. In heterozygotes, the reduced plasminogen activity in plasma seems to be insufficient to prevent thrombosis, which may develop after trauma and is manifested as deep vein thrombosis, thrombophlebitis or pulmonary embolism.

Conventional biological assays for plasminogen activity and antigen concentration do not accurately identify the molecular basis of thrombosis, because plasminogen can be decreased in several acquired disease states, such as liver dysfunction and disseminated intravascular coagulation, or by thrombolytic therapy using plasminogen activators. Because proper therapy is dictated by the nature of the underlying condition, it is important to make a definitive diagnosis in the case of a genetic molecular abnormality. An additional complication in diagnosing plasminogen-related disorders arises from the high degree of homology between plasminogen and apolipoprotein(a). This homology makes it difficult to distinguish between DNA sequences encoding the two proteins.

Previously described methods of identifying the presence of the Thr-601 plasminogen mutation are not well suited to clinical use. Miyata et al. (Proc. Natl. Acad. Sci. USA 79:6132-6136, 1982) used proteolytic digestion of plasminogen and amino acid sequence analysis of the resultant peptides to characterize the mutation. Aoki et al. (Biochemical Genetics 22:871-881, 1984) utilized electrofocusing, zymography and immunofixation of neuraminidase-treated plasminogen. The entire procedure required four or more days to perform.

There is therefore a need in the art for improved methods of detecting the presence of mutations in the plasminogen gene. Such methods should be technically simple and rapid enough to permit clinical use. The present invention provides such methods for genetic diagnosis at the DNA level and has the additional advantage of not being influenced by the presence of other disease conditions.

Disclosure of the Invention

Briefly stated, the present invention is directed toward methods for detecting the presence of a mutation in the plasminogen gene of a patient. In one aspect of the present invention, the method comprises (a) amplifying a portion of genomic DNA from a patient, the portion including a predetermined exon comprising the site of a selected mutation and at least 14 base pairs of each of two intron sequences flanking the exon; (b) exposing the amplified DNA to a restriction endonuclease capable of differentially cleaving DNA having the selected mutation and wild-type plasminogen DNA, under conditions suitable for activity of the endonuclease; and (c) analyzing the exposed DNA to detect the presence or absence of cleavage fragments diagnostic for the selected mutation. Within preferred embodiments, the selected mutation is the Phe-355 mutation or the Thr-601 mutation. The method may also include,

prior to the step of amplifying, isolating genomic DNA from the patient.

Within a related aspect of the present invention, a method of detecting the presence of a mutation in the plasminogen gene of a patient is disclosed, wherein the method generally comprises (a) denaturing genomic DNA from the patient; (b) annealing the denatured genomic DNA to a pair of oligonucleotide primers, wherein the first primer is complementary to a first sequence of at least about fifteen consecutive nucleotides of a first intron on the coding strand of the genomic DNA, and wherein the second primer is complementary to a second sequence of at least about fifteen consecutive nucleotides of a second intron on the noncoding strand of the genomic DNA, the introns flanking the exon comprising the site of a selected mutation; (c) extending the annealed primers to produce double-stranded DNA fragments, the fragments including the site of the selected mutation; (d) denaturing the double-stranded DNA fragments; (e) annealing the denatured DNA fragments to the pair of oligonucleotide primers and extending the annealed primers to produce selectively amplified DNA; (f) exposing the selectively amplified DNA to a restriction endonuclease capable of differentially cleaving DNA having the selected mutation and wild-type plasminogen DNA, under conditions suitable for activity of the endonuclease; and (g) analyzing the exposed DNA to detect the presence or absence of cleavage fragments diagnostic for the selected mutation, wherein the selected mutation is the Phe-355 mutation or the Thr-601 mutation. Within a preferred embodiment, the primers are extended using Taq DNA polymerase.

Within another aspect of the present invention, a diagnostic kit for the rapid detection of the Thr-601 mutation in the plasminogen gene of a patient is disclosed. The kit includes, within suitable compartments: a pair of oligonucleotide primers, the first primer being complementary to a first sequence of at least about fifteen consecu-

tive nucleotides of an intron on the coding strand of genomic DNA from a patient, the second primer being complementary to a second sequence of at least about fifteen consecutive nucleotides of a second intron on the noncoding strand of the genomic DNA, the introns flanking the exon coding for amino acid residue 601 of plasminogen; Taq DNA polymerase; control DNA; a restriction endonuclease capable of differentially cleaving Ala-601 plasminogen DNA and Thr-601 plasminogen DNA; and suitable buffers.

Within yet another aspect of the present invention, a diagnostic kit for the rapid detection of the Phe-355 mutation in the plasminogen gene of a patient is provided. The kit comprises, contained within suitable compartments, (a) a pair of oligonucleotide primers, the first primer being complementary to a first sequence of at least about fifteen consecutive nucleotides of an intron on the coding strand of genomic DNA from a patient, the second primer being complementary to a second sequence of at least about fifteen consecutive nucleotides of a second intron on the noncoding strand of the genomic DNA, the introns flanking the exon coding for amino acid 355 of plasminogen; (b) Taq DNA polymerase; (c) control DNA; (d) a restriction endonuclease capable of differentially cleaving Val-355 plasminogen DNA and Phe-355 plasminogen DNA; and (e) suitable buffers.

These and other aspects of the present invention will become evident upon reference to the following detailed description and attached drawings.

Brief Description of the Drawings

Figure 1 illustrates the cDNA sequence and amino acid sequence of plasminogen. The positions of certain restriction enzyme recognition sites are shown. Numbers in the left margin refer to nucleotide positions. Numbers above the sequence refer to amino acid positions.

Figure 2 illustrates portions of the sequence of the normal human plasminogen gene. N indicates an undeter-

mined nucleotide. Arrows indicate exon-intron bou. Exon sequences are underlined and labeled with numerals. The 5' end of exon I and the 3' end of ex were not determined; the 3' end of exon XIX is shown a proposed polyadenylation signal. The partial gene sequ is presented in 10 sections, labeled a through j, show a, exon I and adjacent intron sequences; b, exons II III and adjacent intron sequences; c, exon IV and adjace intron sequences; d, exon V and adjacent intron sequence e, exon VI and adjacent intron sequences; f, 10,000 base pairs comprising exons VII, VIII, IX and X; g, 10,000 base pairs comprising exons XI, XII and XIII; h, 10,000 base pairs comprising exons XIV, XV, XVI and XVII; i, intron sequence (4473 bp); and j, exons XVIII and XIX with adjacent intron sequences. Nucleotides in each of sections a through j are independently numbered as designated in the right margin, beginning with 1.

Figure 3 illustrates a portion of the genomic DNA sequence encoding plasminogen and the sequences of two sets of oligonucleotide primers (designated A39, 1A, 10A and 11A) used to selectively amplify a portion of the genomic DNA. The locations of certain restriction enzyme recognition sites are indicated.

Figure 4 shows the results of a Fnu 4HI digest of selectively amplified genomic DNAs from three unrelated patients with abnormal plasminogen and a normal individual. The molecular weight marker is a 123-bp ladder obtained from Bethesda Research Laboratories. AbI, II and III refer to samples from abnormal patients I, II and III, respectively.

Best Mode for Carrying Out the Invention

Prior to setting forth the invention, it may be useful to define certain terms used herein.

Selectively amplifying: The process of increasing the copy number of a preselected DNA sequence or

nt relative to the copy number of other sequences or ents in a sample.

Differentially cleaving: Cleaving a first uence or set of sequences but not cleaving a second quence or set of sequences. Restriction endonucleases fferentially cleave DNA sequences due to their ability to pecifically recognize short stretches of paired bases, frequently palindromic sequences of four to six base pairs. Cleavage may occur within the recognition sequence or at some specific distance away from the recognition sequence.

Site of the selected mutation: The position in a gene at which a mutation is known to occur, regardless of whether that particular allele carries the mutant or wild-type sequence at the site.

As noted above, reduced plasminogen activity can lead to thrombotic episodes. Also as noted above, such a reduction in activity can result from a variety of causes, including genetic abnormalities. Practical methods of clinical screening for genetic abnormalities in plasminogen have heretofore been unavailable.

The present invention provides methods useful in diagnosing cases of thrombosis, in genetic screening and in prenatal diagnosis. The methods are simple, rapid, and do not require the use of radioactive isotopes, so are particularly useful in many clinical laboratories that lack in the special facilities necessary for handling radioisotopes.

The present invention is related, in part, to the elucidation of the human plasminogen gene sequence, portions of which are shown in Figures 2 and 3. Knowledge of this sequence has permitted the design of oligonucleotide primers that may be used to selectively amplify those portions of the gene encoding amino acid residue 601 or amino acid residue 355. In a similar manner, other abnormal plasminogen gene sequences may be analyzed, allowing those skilled in the art to selectively amplify exons comprising sites of other selected mutations.

The methods of the present invention are applied to genomic DNA samples from a patient. In one embodiment, the genomic DNA is first isolated, using conventional procedures. A convenient source of isolated genomic DNA is leukocytes, which may be readily obtained from a small (e.g., 10 ml) blood sample. Other cell types may also be used. DNA may be isolated from leukocytes using the technique of Bell et al. (Proc. Natl. Acad. Sci. USA 78:5759-5763, 1981). Briefly, blood is collected in the presence of an anticoagulant, the cells are lysed, and the nuclei are collected. The nuclei are then treated with sodium dodecyl sulfate and proteinase K and the DNA is extracted from the mixture with phenol/chloroform/isoamyl alcohol. The DNA is then precipitated and resuspended in a suitable buffer, such as 10 mM Tris-HCl (pH 7.5), 1 mM EDTA. Alternatively, by using the method disclosed disclosed by Kogan et al. (New Eng. J. Med. 317:985-990, 1987), the methods of the present invention may be applied directly to tissue samples, without the need to isolate the DNA. For example, chorionic villus samples can be screened directly by disrupting the tissue by vortexing in a solution of 0.1M NaOH, 2M NaCl, 0.5% SDS. The sample is then boiled for two minutes, centrifuged, and an aliquot is taken for amplification. This facilitates the application of these methods to prenatal diagnosis of the plasminogen abnormality.

Genomic DNA (either isolated or in the form of a suitable tissue sample) is then selectively amplified to provide a high copy number of the desired portion of the plasminogen gene (e.g., the portion encoding amino acid residue 601 or the portion encoding amino acid residue 355). Preferably, a sequence of approximately 200-1,000, most preferably about 300-400, base pairs is selectively amplified. In a preferred embodiment, the exon encoding amino acid 601 and portions of the intron sequences flanking this exon are selectively amplified. Similarly, the exon encoding amino acid 355 and portions of the flanking introns may be selectively amplified. A preferred method

of amplification is the polymerase chain reaction, described by Mullis (U.S. Patent Nos. 4,683,202 and 4,683,195). Briefly, the genomic DNA is denatured to separate the coding and noncoding strands. Denaturation is preferably accomplished by heat treatment of the DNA, generally treatment at about 80°C-105°C for about one to ten minutes, although enzymatic denaturation may also be used. Most preferably, the DNA is heated at about 93°C for one minute. The denatured DNA is then combined with a molar excess of a pair of oligonucleotide primers under conditions which allow the DNA strands to anneal to the primers (e.g., 60°C for one to three minutes, preferably about two minutes). Preferably, each primer is used at a concentration of about 1μM for amplification of one microgram of genomic DNA. Suitable results may be obtained with 5μg of primer per μg of target DNA. One of the primers is complementary to a sequence on the coding strand and the second primer is complementary to a sequence on the noncoding strand, the sequences flanking the region to be amplified. "Sequences flanking the region to be amplified" include exon sequences, sequences of introns immediately adjacent to the exon to be amplified and sequences of other introns, so long as the amplified region includes the site of the selected mutation. The flanking sequences should be selected so as to provide an amplified portion of the gene within the size limits noted above. Although 100% complementarity is not required, a high degree of complementarity of primer and genomic DNA is advantageous in that it results in high specificity and efficiency of amplification. For use within the present invention, the primers must be sufficiently complementary to hybridize with their respective strands on the genomic DNA. The annealed primers are enzymatically extended using a DNA polymerase and all four deoxyribonucleotide triphosphates (dNTP's). Suitable polymerases include E. coli DNA polymerase I, the Klenow fragment of E. coli DNA polymerase I, Taq DNA polymerase, and T4 DNA polymerase. Taq DNA polymerase (Saiki et al.,

Science 239:487-491, 1988) is particularly preferred. The reaction mixture is incubated under conditions of time and temperature suitable for the activity of the polymerase. When using the Taq DNA polymerase the mixture is incubated at about 70°C ± 10°C for approximately three minutes. As will be appreciated by one skilled in the art, the exact time and temperature will be determined by the melting point of the annealed DNA. The resulting extension products are separated from the original DNA strands, preferably by heat denaturation. The annealing, extension and separation steps are then repeated, preferably about 25 to 30 times, until the desired degree of amplification is obtained. At that time, the final separation step is omitted, and double-stranded DNA is isolated. In general, it is preferred to add the primers and dNTP's at the beginning of the amplification reaction in sufficient quantity to allow full amplification to occur without the need to add additional reagents during the course of the reaction series. The use of Taq DNA polymerase facilitates such a process, as this heat-stable enzyme is not inactivated by the heat denaturation steps and the reaction need not be interrupted for the addition of more polymerase.

As noted above, oligonucleotide primers for use in the polymerase chain reaction are constructed to be complementary to sequences flanking an exon comprising the site of a selected mutation, such as the exon containing the codon for amino acid 601 or the exon containing the codon for amino acid 355. A first primer is designed to be complementary to a sequence on the coding strand, and a second primer is complementary to a sequence on the noncoding strand of the DNA. Preferably, the primers will be complementary to intron sequences because intron sequences will exhibit the least amount of intergene homology. The primers are preferably at least about 15-20 bases in length, more preferably at least about 25 bases in length. Primers shorter than about 20 bases will often have reduced specificity, and may anneal to and amplify

unwanted sequences. Primers are preferably less than 50 bases in length, more preferably less than about 30 bases in length. Longer primers may self-anneal or their use may lead to reduced specificity.

Within the present invention, alternative methods of DNA amplification may also be used. For example, a genomic library may be prepared by digesting genomic DNA from a patient and cloning the resultant DNA fragments into a suitable vector (e.g., plasmid, cosmid or bacteriophage). The library is then amplified by conventional methods, and plasminogen-encoding clones are screened for the presence of the mutation.

The amplified DNA is then incubated with a restriction endonuclease which is capable of differentially cleaving normal and abnormal plasminogen DNA. Suitable restriction endonucleases for identification of the Thr-601 mutation include Fnu 4HI and Bbv I. Endonucleases suitable for identification of the Phe-355 mutation include Ava II, Bam NxI, Cau I (Bingham and Darbyshire, Gene 18:87-91, 1982; Molemans et al., Gene 18:93-96, 1982), Hgi BI, Hgi CII, Hgi EI and Sau 96I. However, the invention is not limited to the use of particular enzymes, but is intended to include the use of other suitable enzymes which may from time to time become available. Restriction endonucleases are commercially available from, for example, New England Biolabs (Beverly, Mass.), Bethesda Research Laboratories (Gaithersburg, Md.) and other suppliers. The amplified DNA is incubated with the endonuclease under conditions of time, temperature and buffer composition suitable for the activity of the endonuclease. Such conditions are generally specified by the supplier.

Following exposure to the restriction endonuclease, the DNA sample is analyzed to detect the presence or absence of cleavage fragments diagnostic for the selected mutation, for example by electrophoretic separation of DNA fragments. In a preferred embodiment, the DNA is electrophoresed on an agarose gel containing ethidium

bromide. Endonuclease Fnu 4HI cleaves the normal plasminogen sequence at the codon for Ala-601. The presence of the Thr-601 mutation prevents this cleavage, resulting in no change in fragment size following exposure to the enzyme. Priming in the introns flanking the codon for amino acid 601 as disclosed in more detail below resulted in amplification of a ~340 bp fragment. The normal sequence could be cleaved by Fnu 4HI to yield fragments of about 240 bp and 100 bp. Also, as discussed in more detail below, the mutation of Val-355 to Phe can be detected by amplifying a ~390 bp fragment, digesting the amplified DNA with Ava II and analyzing the digested DNA. The Phe-355 mutation results in the presence of a 360 bp fragment, which is not present in the Ava II digest of wild-type DNA.

The methods described herein are well suited to clinical use. In particular, the combination of the polymerase chain reaction and restriction analysis can be used to diagnose the specific plasminogen abnormality at the DNA level in a rapid and straightforward manner. Partial purification of genomic DNA from leukocytes takes several hours, and amplification by the polymerase chain reaction takes about three hours. Restriction digestion of the amplified DNA and its analysis on agarose gels require about one hour or less each. Therefore, the entire diagnostic procedure can be performed in a single day.

As briefly described above, suitable kits for diagnosing these plasminogen mutations contain oligonucleotide primers, Taq DNA polymerase, an appropriate restriction enzyme, buffers, and normal (control) DNA in appropriate packaging.

The following examples are offered by way of illustration, and not by way of limitation.

EXPERIMENTAL

Taq DNA polymerase was obtained from New England Biolabs and The Perkin Elmer Corporation (Norwalk, Conn.).

Restriction endonucleases and T4 DNA ligase were purchased from Bethesda Research Laboratories (Gaithersburg, Md.) or New England Biolabs. The Klenow fragment of Escherichia coli DNA polymerase, bacterial alkaline phosphatase, ATP, deoxynucleotides, dideoxynucleotides, M13mp18, and M13mp19 were supplied by Bethesda Research Laboratories. dATP[α-^{35}S] was provided by Amersham (Chicago, Ill.).

Oligonucleotides were synthesized using a nucleotide synthesizer (Applied Biosystems, Foster City, Calif.) and kindly supplied by Drs. Patrick S.H. Chou, Yim Foon Lee and Jeff Harris.

Example 1

Leukocyte genomic DNA samples were obtained from three unrelated Japanese patients with abnormal plasminogen (named abnormal I, II and III, respectively), a daughter of abnormal III (abnormal III-2) and three unrelated normal American white individuals. Abnormals I, II and III-2 had a history of thrombosis, but abnormal III did not. The plasma of abnormal I had a trace of plasminogen activity in spite of a normal plasminogen antigen concentration, and the plasma from the mother and a sister of abnormal I showed a 50% reduction in enzymatic activity of plasminogen. Accordingly, abnormal I is a homozygote of a nonfunctional plasminogen variant. Abnormal II is a heterozygote of a plasminogen variant, since the plasminogen in the plasma of the patient and his two daughters has about half of the specific activity (activity per antigen) of normal plasminogen. Abnormal III is a homozygote of the plasminogen variant named PLG B (Nishimukai et al., Hum. Hered. 36:137-142, 1986) as determined by isoelectric focusing. Abnormal III-2 is a heterozygote of PLG B with a normal plasminogen concentration and half of normal specific activity.

Genomic DNA samples were prepared from the leukocytes of the patients with abnormal plasminogen and from normal individuals by the method of Bell et al. (ibid.). Typically, 10-40 ml of blood is collected in citrate buffer.

Ten ml of blood is added to 90 ml of 0.32 M sucrose, 10 mM Tris pH7.5, 5mM $MgCl_2$, 1% Triton X-100, and the mixture is incubated at 4°C to lyse the cells. Nuclei are collected by centrifugation at 1,000 x g for 10 minutes and resuspended in 4.5 ml of 0.075 M NaCl, 0.024 M EDTA, pH 8.0. The nuclei are treated with SDS and proteinase K, and the DNA is extracted with chloroform/phenol/isoamyl alcohol, precipitated with ethanol and resuspended in the appropriate buffer.

Nucleotide primers A39 and 1A (Figure 3) for the putative introns N and O flanking the exon coding for the amino acid residue-601 of plasminogen (exon XV) were synthesized for the polymerase chain reaction. These regions were selected because they lie outside the putative exon 15, and upon selective amplification they produce a fragment of a length suitable for analysis by restriction digestion and DNA sequencing. Both the 5'- and 3'-ends were modified to generate convenient restriction sites (Hind III) for cloning directly into the M13 sequencing vector. One µg of genomic DNA was amplified in a 100 µl reaction mixture containing 50 mM KCl, 10 mM Tris (pH 8.4), 2.5 mM $MgCl_2$, each primer (A39 and 1A, Figure 3) at 1 µM, each dNTP at 200 µM, gelatin at 200 µg/ml, and 2.5 units of Taq DNA polymerase (Saiki et al., Science 239:487-491, 1988). The sample was placed in a small Eppendorf tube and overlaid with 100 µl of mineral oil to prevent evaporation. The sample was heated at 93°C for one minute to denature the DNA, cooled to 60°C for two minutes to anneal the primers, and incubated at 70°C for three minutes to extend the annealed primers. The procedure was repeated for a total of 25-30 cycles of amplification. At the end of the last cycle, the sample was incubated at 70°C for 7 minutes to ensure the completion of the final extension step. After precipitation with ethanol and resuspension in 100 µl TE buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA), 5 µl was applied to a 1.5% agarose gel for submerged electrophoresis, and stained with ethidium bromide. A discrete band of

about 340 bp was obtained for each sample, as predicted from the sequence of the gene for normal plasminogen.

The samples from abnormals I, II, III, III-2 and normal individuals were digested with three units of Fnu 4HI endonuclease for one hour or with six units of enzyme for four hours at 37°C. Five microliters of each sample was then applied to a 1.5% agarose gel containing ethidium bromide. The 340 bp fragment of normal DNA was cleaved into two fragments (about 240 and 100 bp), while that of the DNA from abnormal III remained unchanged (Figure 4). The Fnu 4HI digests of the 340 bp fragments from abnormals II and III-2 each showed a mixed pattern of normal DNA and the DNA from abnormal III. In contrast, the DNA from abnormal I was cleaved completely. Prolonged digestion of the samples for four hours with six units of enzyme gave exactly the same results (Figure 4). The amplification and digestion of the genomic DNAs from abnormals I, II, III and III-2 was performed eight, two, three and two times, respectively, and the results obtained were the same in each experiment for each sample. Fnu 4HI recognizes only the GCNGC sequence, suggesting that one or more of these four nucleotides in the DNAs of abnormals III, III-2, and II is replaced by other nucleotides. Alternatively, a short stretch of nucleotides could be deleted or inserted in the abnormal DNA.

To characterize the mutation(s) at the DNA level, the amplified fragments were sequenced. Since both the 5'- and 3'-end primers were designed to produce double-stranded fragments flanked by Hind III recognition sequences, the amplified 340 bp fragments from normal and abnormal individuals were digested with Hind III and ligated into M13 sequencing vectors cut with Hind III. In order to obtain the DNA sequence coding for the specific region around amino acid residue 601, the amplified DNAs were also digested with Hinc II and Pst I endonucleases. The digested samples were electrophoresed on a 1.5% agarose gel, electroeluted, and dialyzed against 0.1X TBE (1X TBE

is 89 mM Tris-borate, 89 mM boric acid, 20 mM EDTA) overnight. The dialyzed samples were extracted with phenol and chloroform, precipitated with ethanol, resuspended in TE, and finally subcloned into M13mp18 or mp19 in order to obtain discrete overlapping sequences. The DNA sequences of the inserts were then obtained using the dideoxynucleotide method (Sanger et al. Proc. Natl. Acad. Sci. USA 74:5463-5467, 1977) with dATP [α-^{35}S] and buffer gradient gels (Biggin et al. Proc. Natl. Acad. Sci. USA 80:3963-3965, 1983).

The DNA sequences obtained from the three normal individuals included 343 bp. These sequences were the same as expected for the normal gene except for the presence of Hind III sites at both the 5'- and 3'ends. The sequence of the Hinc II-Pst I fragments from the normal DNAs included 205 bp, and was also the same as the established sequence of the normal gene for plasminogen. The actual sequence of the region coding for amino acid 601 (Ala) included ACTGCTGC in the normal gene.

On the other hand, the DNA sequence analysis of both Hind III and Hinc II-Pst I fragments of abnormal III revealed that the gene of abnormal III contained the sequence ACTACTGC. This corresponds to a single base change resulting in the substitution of Thr (ACT) for Ala (GCT). Twenty-three templates from the amplified samples of abnormal III were sequenced and all of them showed the same abnormal sequence (G to A change). No other alterations of nucleotides were found by DNA sequence analysis.

When twelve templates for abnormal II were sequenced, one-half of them showed the same sequence as the normal gene except for a point mutation (T to C) 5 nucleotides prior to the Fnu 4HI site, and the other half had the same abnormal sequence as abnormal III. These results confirmed that abnormal III is a homozygote of a plasminogen variant and that abnormal II is a heterozygote of the same variant allele.

The exon XV DNA sequence of abnormal I was the same as that of the normal gene, indicating that the abnormality in this molecule is in another region.

A second set of primers (designated 10A and 11A in Figure 3), flanked by Eco RI recognition sequences and four additional nucleotides, was used to confirm the results. A band of 360 bp was obtained for each sample as predicted.

Example 2

Plasminogen gene exon X DNA of abnormal I was amplified essentially as described above using primers K4a-5' (5' GTC AGA ATT CTC AGA GGC TAC CGT ACT 3'; coding strand primer) and K4a-3' (5' CTA CGA ATT CTG GCT CTA ACA CAA ATT TCC 3'; noncoding strand primer). The amplified DNA was digested with Eco RI, and the resulting ~390 bp fragment was cloned into an M13 phage vector and sequenced. Sequence analysis revealed the presence of the sequence GTGTTCCAG in six of the templates, as compared to the wild-type sequence GTGGTCCAG. This T for G substitution results in the substitution of a phenylalanine residue for the normal valine residue at amino acid position 355, located several residues upstream of Kringle 4 in the A chain (Figure 1).

DNA samples from normal and abnormal individuals were digested with five units of Ava II endonuclease for one hour at 37°C. The 390 bp DNA fragment from the normal individuals was cleaved into three fragments of approximately 230 bp, 130 bp and 30 bp. DNA samples from abnormal I and two daughters (abnormals I-2 and I-3) and a nephew (abnormal I-4) of abnormal I showed a mixture of 360 bp, 230 bp, 130 bp and 30 bp fragments. These results indicated that the abnormal patients were heterozygous for the Phe-355 mutation. Thus, this mutation can be diagnosed by the presence of a 360 bp Ava II fragment when DNA is selectively amplified using primers K4a-5' and K4a-3'.

In a second series of experiments, DNA from abnormals I, I-2, I-3 and I-4 was amplified using primers K4a-5' and K4a-32 (5' AAA TGA ATT CCT AGG AAG TTG GCT TGA AGC 3'; noncoding strand primer). Digestion of the resulting ~370 bp fragment with Ava II confirmed the loss of an Ava II site in the abnormal DNA, and also confirmed the diagnosis of abnormals I, I-2, I-3 and I-4 as heterozygotes of the Phe-355 mutation.

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

Claims

1. A method of detecting the presence of a mutation in the plasminogen gene of a patient, comprising:

amplifying a portion of genomic DNA from the patient, said portion including a predetermined exon comprising the site of a selected mutation and at least 14 base pairs of each of two intron sequences flanking said predetermined exon;

exposing said amplified DNA to a restriction endonuclease capable of differentially cleaving DNA having the selected mutation and wild-type plasminogen DNA under conditions suitable for activity of the endonuclease; and

analyzing the exposed DNA to detect the presence or absence of cleavage fragments diagnostic for the selected mutation.

2. The method of claim 1 wherein the selected mutation is the Phe-355 mutation or the Thr-601 mutation.

3. A method of detecting the presence of a mutation in the plasminogen gene of a patient, comprising:

a. denaturing genomic DNA from the patient;

b. annealing the denatured genomic DNA to a pair of oligonucleotide primers, wherein the first primer is complementary to a first sequence of at least about fifteen consecutive nucleotides of a first intron on the coding strand of the genomic DNA, and wherein the second primer is complementary to a second sequence of at least about fifteen consecutive nucleotides of a second intron on the noncoding strand of the genomic DNA, wherein said introns flank the exon comprising the site of a selected mutation;

c. extending the annealed primers to produce double-stranded DNA fragments, said fragments including the site of the selected mutation;

d. denaturing the double-stranded DNA fragments;

e. annealing the denatured DNA fragments to the pair of oligonucleotide primers and extending the annealed primers to produce selectively amplified DNA;

f. exposing said selectively amplified DNA to a restriction endonuclease capable of differentially cleaving DNA having the selected mutation and wild-type plasminogen DNA, under conditions suitable for activity of the endonuclease; and

g. analyzing the exposed DNA to detect the presence or absence of cleavage fragments diagnostic for the selected mutation, wherein the selected mutation is the Phe-355 mutation or the Thr-601 mutation.

4. The method of claim 3 wherein the primers are extended using Taq DNA polymerase.

5. The method of claim 3 wherein each of said first and second primers is from about twenty to about thirty nucleotides in length, inclusive.

6. The method of claim 3 wherein said selected mutation is the Thr-601 mutation and said first primer includes the sequence CAA TTT AAC TAA AAT TTG AAC TAA AT or TGT ACA ATG GAG CAG AAC AAA.

7. The method of claim 3 wherein said selected mutation is the Thr-601 mutation and said second primer includes the sequence TCA TGT CTA CTA AAA CAC CCG GAC TTA or TCT CCT TTC TGT GTC ATG TCT A.

8. The method of claim 3 wherein said selected mutation is the Phe-355 mutation and said first primer includes the sequence GTC AGA ATT CTC AGA GGC TAC CGT ACT.

9. The method of claim 3 wherein said selected mutation is the Phe-355 mutation and said second primer includes the sequence CTA CGA ATT CTG GCT CTA ACA CAA ATT TCC or AAA TGA ATT CCT AGG AAG TTG GCT TGA AGC.

10. The method of claim 3, further comprising the step of isolating genomic DNA from the patient prior to the step of denaturing the genomic DNA.

11. The method of claim 3 wherein the endonuclease differentiates between G and A in the first position of the codon for amino acid 601 of plasminogen.

12. The method of claim 11 wherein the restriction endonuclease is selected from the group consisting of Fnu 4HI and Bbv I.

13. The method of claim 3 wherein said endonuclease differentiates between G and T in the first position of the codon for amino acid 355 of plasminogen.

14. The method of claim 13 wherein the restriction endonuclease is selected from the group consisting of Ava II and Sau 96I.

15. The method of claim 3 wherein the steps of denaturing comprise heat treatment of the DNA.

16. The method of claim 3 wherein approximately 300-400 bp of genomic DNA is amplified.

17. The method of claim 3 wherein steps d and e are repeated in sequence from about twenty-three to about twenty-eight times prior to step f.

18. A diagnostic kit for the rapid detection of the Thr-601 mutation in the plasminogen gene of a patient, comprising in suitable compartments within the kit:

a pair of oligonucleotide primers, the first primer being complementary to a first sequence of at least about fifteen consecutive nucleotides of an intron on the coding strand of genomic DNA from a patient, the second primer being complementary to a second sequence of at least about fifteen consecutive nucleotides of a second intron on the noncoding strand of the genomic DNA, the introns flanking the exon coding for amino acid residue 601 of plasminogen;

Taq DNA polymerase;

control DNA;

a restriction endonuclease capable of differentially cleaving Ala-601 plasminogen DNA and Thr-601 plasminogen DNA; and

suitable buffers.

19. A diagnostic kit for the rapid detection of the Phe-355 mutation in the plasminogen gene of a patient, comprising in suitable compartments within the kit:

a pair of oligonucleotide primers, the first primer being complementary to a first sequence of at least about fifteen consecutive nucleotides of an intron on the coding strand of genomic DNA from a patient, the second primer being complementary to a second sequence of at least about fifteen consecutive nucleotides of a second intron on the noncoding strand of the genomic DNA, the introns flanking the exon coding for amino acid 355 of plasminogen;

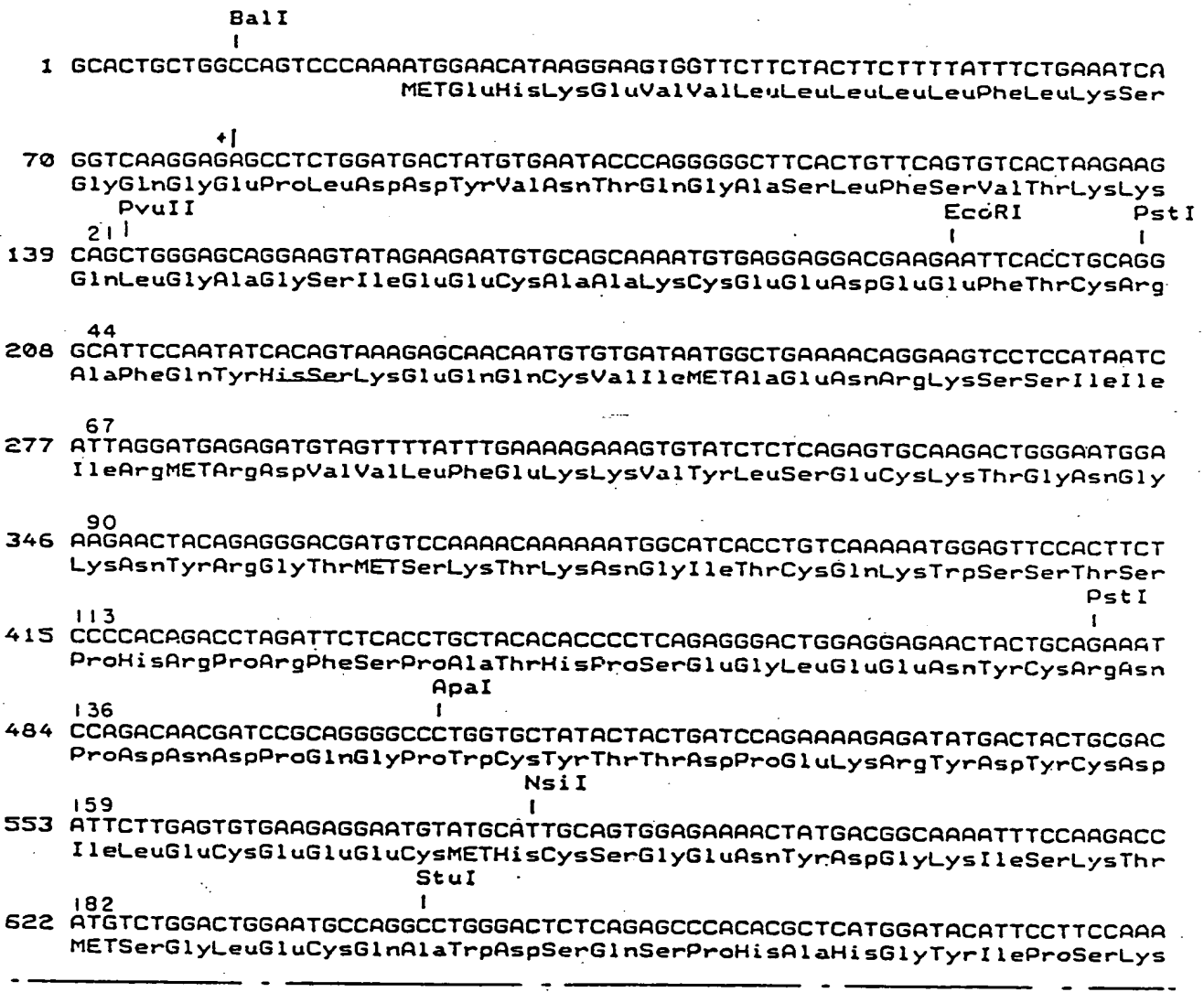
Taq DNA polymerase;

control DNA;

a restriction endonuclease capable of differentially cleaving Val-355 plasminogen DNA and Phe-355 plasminogen DNA; and

suitable buffers.

FIG. 1


BalI
1 GCACTGCTGGCCAGTCCCAAAATGGAACATAAGGAAGTGGTTCTTCTACTTCTTTTATTTCTGAAATCA
METGluHisLysGluValValLeuLeuLeuLeuLeuPheLeuLysSer
70 GGTCAAGGAGAGCCTCTGGATGACTATGTGAATACCCAGGGGGCTTCACTGTTCAGTGTCACTAAGAAG
GlyGlnGlyGluProLeuAspAspTyrValAsnThrGlnGlyAlaSerLeuPheSerValThrLysLys
PvuII
EcoRI
PstI
139 CAGCTGGGAGCAGGAAGTATAGAAGAATGTGCAGCAAAATGTGAGGAGGACGAAGAATTCACCTGCAGG
GlnLeuGlyAlaGlySerIleGluGluCysAlaAlaLysCysGluGluAspGluGluPheThrCysArg
208 GCATTCCAATATCACAGTAAAGAGCAACAATGTGTGATAATGGCTGAAAACAGGAAGTCCTCCATAATC
AlaPheGlnTyrHisSerLysGluGlnGlnCysValIleMETAlaGluAsnArgLysSerSerIleIle
277 ATTAGGATGAGAGATGTAGTTTTATTTGAAAAGAAAGTGTATCTCTCAGAGTGCAAGACTGGGAATGGA
IleArgMETArgAspValValLeuPheGluLysLysValTyrLeuSerGluCysLysThrGlyAsnGly
346 AAGAACTACAGAGGGACGATGTCCAAAACAAAAAATGGCATCACCTGTCAAAAATGGAGTTCCACTTCT
LysAsnTyrArgGlyThrMETSerLysThrLysAsnGlyIleThrCysGlnLysTrpSerSerThrSer
PstI
415 CCCCACAGACCTAGATTCTCACCTGCTACACACCCCTCAGAGGGACTGGAGGAGAACTACTGCAGAAAT
ProHisArgProArgPheSerProAlaThrHisProSerGluGlyLeuGluGluAsnTyrCysArgAsn
ApaI
484 CCAGACAACGATCCGCAGGGGCCCTGGTGCTATACTACTGATCCAGAAAAGAGATATGACTACTGCGAC
ProAspAsnAspProGlnGlyProTrpCysTyrThrThrAspProGluLysArgTyrAspTyrCysAsp
NsiI
553 ATTCTTGAGTGTGAAGAGGAATGTATGCATTGCAGTGGAGAAAACTATGACGGCAAAATTTCCAAGACC
IleLeuGluCysGluGluGluCysMETHisCysSerGlyGluAsnTyrAspGlyLysIleSerLysThr
StuI
622 ATGTCTGGACTGGAATGCCAGGCCTGGGACTCTCAGAGCCCACACGCTCATGGATACATTCCTTCCAAA
METSerGlyLeuGluCysGlnAlaTrpAspSerGlnSerProHisAlaHisGlyTyrIleProSerLys

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    205
691 TTTCCAAACAAGAACCTGAAGAAGAATTACTGTCGTAACCCCGAGAGGGAGCTGCGGCCTTGGTGTTTC
    PheProAsnLysAsnLeuLysLysAsnTyrCysArgAsnProGluArgGluLeuArgProTrpCysPhe
                          Eco47III
    228                   |
760 ACCACCGACCCCAACAAGCGCTGGGAACTTTGTGACATCCCCCGCTGCACAACACCTCCACCATCTTCT
    ThrThrAspProAsnLysArgTrpGluLeuCysAspIleProArgCysThrThrProProProSerSer

    251
829 GGTCCCACCTACCAGTGTCTGAAGGGAACAGGTGAAAACTATCGCGGGAATGTGGCTGTTACCGTGTCC
    GlyProThrTyrGlnCysLeuLysGlyThrGlyGluAsnTyrArgGlyAsnValAlaValThrValSer
                              ApaLI
    274                       |
898 GGGCACACCTGTCAGCACTGGAGTGCACAGACCCCTCACACACATAACAGGACACCAGAAAACTTCCCC
    GlyHisThrCysGlnHisTrpSerAlaGlnThrProHisThrHisAsnArgThrProGluAsnPhePro
                                                  ApaI NcoI
    297                                           |  ||
967 TGCAAAAATTTGGATGAAAACTACTGCCGCAATCCTGACGGAAAAAGGGCCCCATGGTGCCATACAACC
    CysLysAsnLeuAspGluAsnTyrCysArgAsnProAspGlyLysArgAlaProTrpCysHisThrThr
                              ScaI
    320                       |
1036 AACAGCCAAGTGCGGTGGGAGTACTGTAAGATACCGTCCTGTGACTCCTCCCCAGTATCCACGGAACAA
    AsnSerGlnValArgTrpGluTyrCysLysIleProSerCysAspSerSerProValSerThrGluGln
                                                      NcoI
    343                                               |  360          365
1105 TTGGCTCCCACAGCACCACCTGAGCTAACCCCTGTGGTCCAGGACTGCTACCATGGTGATGGACAGAGC
    LeuAlaProThrAlaProProGluLeuThrProValValGlnAspCysTyrHisGlyAspGlyGlnSer

    366
1174 TACCGAGGCACATCCTCCACCACCACCACAGGAAAGAAGTGTCAGTCTTGGTCATCTATGACACCACAC
    TyrArgGlyThrSerSerThrThrThrThrGlyLysLysCysGlnSerTrpSerSerMETThrProHis
                                                          PstI
    389                                                   |
1243 CGGCACCAGAAGACCCCAGAAAACTACCCAAATGCTGGCCTGACAATGAACTACTGCAGGAATCCAGAT
    ArgHisGlnLysThrProGluAsnTyrProAsnAlaGlyLeuThrMETAsnTyrCysArgAsnProAsp
                                                      ScaI
    412                                               |
1312 GCCGATAAAGGCCCCTGGTGTTTTACCACAGACCCCAGCGTCAGGTGGGAGTACTGCAACCTGAAAAAA
    AlaAspLysGlyProTrpCysPheThrThrAspProSerValArgTrpGluTyrCysAsnLeuLysLys

    435
1381 TGCTCAGGAACAGAAGCGAGTGTTGTAGCACCTCCGCCTGTTGTCCTGCTTCCAGATGTAGAGACTCCT
    CysSerGlyThrGluAlaSerValValAlaProProProValValLeuLeuProAspValGluThrPro

    458
1450 TCCGAAGAAGACTGTATGTTTGGGAATGGGAAAGGATACCGAGGCAAGAGGGCGACCACTGTTACTGGG
    SerGluGluAspCysMETPheGlyAsnGlyLysGlyTyrArgGlyLysArgAlaThrThrValThrGly

    481
1519 ACGCCATGCCAGGACTGGGCTGCCCAGGAGCCCCATAGACACAGCATTTTCACTCCAGAGACAAATCCA
    ThrProCysGlnAspTrpAlaAlaGlnGluProHisArgHisSerIlePheThrProGluThrAsnPro

    504
1588 CGGGCGGGTCTGGAAAAAAATTACTGCCGTAACCCTGATGGTGATGTAGGTGGTCCCTGGTGCTACACG
    ArgAlaGlyLeuGluLysAsnTyrCysArgAsnProAspGlyAspValGlyGlyProTrpCysTyrThr

    527
1657 ACAAATCCAAGAAAACTTTACGACTACTGTGATGTCCCTCAGTGTGCGGCCCCTTCATTTGATTGTGGG
    ThrAsnProArgLysLeuTyrAspTyrCysAspValProGlnCysAlaAlaProSerPheAspCysGly
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FIG. 1 CONT.

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                                                                  550
1726 AAGCCTCAAGTGGAGCCGAAGAAATGTCCTGGAAGGGTTGTAGGGGGGTGTGTGGCCCACCCACATTCC
     LysProGlnValGluProLysLysCysProGlyArgValValGlyGlyCysValAlaHisProHisSer
                                                                      EcoRV
                                                                        |
                                                                  573
1795 TGGCCCTGGCAAGTCAGTCTTAGAACAAGGTTTGGAATGCACTTCTGTGGAGGCACCTTGATATCCCCA
     TrpProTrpGlnValSerLeuArgThrArgPheGlyMETHisPheCysGlyGlyThrLeuIleSerPro
                                                          StuI
                                                           |
                                596                601
1864 GAGTGGGTGTTGACTGCTGCCCACTGCTTGGAGAAGTCCCCAAGGCCTTCATCCTACAAGGTCATCCTG
     GluTrpValLeuThrAlaAlaHisCysLeuGluLysSerProArgProSerSerTyrLysValIleLeu
       ApaLI
                                619
1933 GGTGCACACCAAGAAGTGAATCTCGAACCGCATGTTCAGGAAATAGAAGTGTCTAGGCTGTTCTTGGAG
     GlyAlaHisGlnGluValAsnLeuGluProHisValGlnGluIleGluValSerArgLeuPheLeuGlu

                                642
2002 CCCACACGAAAAGATATTGCCTTGCTAAAGCTAAGCAGTCCTGCCGTCATCACTGACAAAGTAATCCCA
     ProThrArgLysAspIleAlaLeuLeuLysLeuSerSerProAlaValIleThrAspLysValIlePro

                                665
2071 GCTTGTCTGCCATCCCCAAATTATGTGGTCGCTGACCGGACCGAATGTTTCATCACTGGCTGGGGAGAA
     AlaCysLeuProSerProAsnTyrValValAlaAspArgThrGluCysPheIleThrGlyTrpGlyGlu

                                688
2140 ACCCAAGGTACTTTTGGAGCTGGCCTTCTCAAGGAAGCCCAGCTCCCTGTGATTGAGAATAAAGTGTGC
     ThrGlnGlyThrPheGlyAlaGlyLeuLeuLysGluAlaGlnLeuProValIleGluAsnLysValCys

                                711
2209 AATCGCTATGAGTTTCTGAATGGAAGAGTCCAATCCACCGAACTCTGTGCTGGGCATTTGGCCGGAGGC
     AsnArgTyrGluPheLeuAsnGlyArgValGlnSerThrGluLeuCysAlaGlyHisLeuAlaGlyGly

                                734
2278 ACTGACAGTTGCCAGGGTGACAGTGGAGGTCCTCTGGTTTGCTTCGAGAAGGACAAATACATTTTACAA
     ThrAspSerCysGlnGlyAspSerGlyGlyProLeuValCysPheGluLysAspLysTyrIleLeuGln
                                      ApaLI
                                757     |
2347 GGAGTCACTTCTTGGGGTCTTGGCTGTGCACGCCCCAATAAGCCTGGTGTCTATGTTCGTGTTTCAAGG
     GlyValThrSerTrpGlyLeuGlyCysAlaArgProAsnLysProGlyValTyrValArgValSerArg

                                780                                 791
2416 TTTGTTACTTGGATTGAGGGAGTGATGAGAAATAATTAATTGGACGGGAGACAGAGTGACGCACTGACT
     PheValThrTrpIleGluGlyValMETArgAsnAsn
                                                          SphI
                                                           |
2485 CACCTAGAGGCTGGAACGAGGGTAGGGATTTAGCATGCTGGAAATAACTGGCAGTAATCAAACGAAGAC

2554 ACTGTCCCCAGCTACCAGCTACGCCAAACCTCGGCATTTTTTGTGTTATTTTCTGACTGCTGGATTCTG

2623 TAGTAAGGTGACATAGCTATGACATTTGTTAAAAATAAACTCTGTACTTAACTTTGA
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FIG. 1 CONT.

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GAATTCCGCA GACATTCCAC CCAAGACCAT TGGGCTCCCA CCTCTACTCT TTTGCCAGTT   60
AATGAATAGG CAGGAATTTC ACTGCCTGGA AAGAGGAACA ATGCTTTCTG GTCCTTATTT  120
CACATCTAAA ATAGAGAGGT CAATTGATTT ATTCCTAAAT ATCTTTGAAC ACTAAAATAG  180
AAGTTTTACA GCATATATAC TACCTGGTTG CTCTAGACTT AAGCCAGGGA AAAGTACAGA  240
TTCAACATTT AAAATTGAGA TAGACGCTTT CCACTTAATG CTACCAGTCT TGCTTTATTT  300
CATGAGAATG AGAATATAAT AATATGGCAT ACGTTCATTT GGGGGAAAGA TTGATGTCTT  360
ATAACATAAT TTATAATTAC AGAAAACATG TGAGTTCACT GGGAATAAAT AAATTTTGAA  420
GATAATAAGA TACTTTCACT TATGTCATAA TTTCTATGTC ATTTGGTGTA GGATGTAGAG  480
ATATTAACGT TTACACCTAA CTCAAGTTTG TCATCTAAGA CCTGAAAGGG TTTTGTCTAT  540
CAGCTGCACC CCTGGGTAGA GACACAACCT TGGGGAAGGC CTCAGCCCCA TCCCTCGTAC  600
AGCAGGAATG AGAACAGCCC TGCCTGTTGG GAAGCTTGAG GGAGGCTATG GACGTGCAGC  660
GCTTGGCAGA AGGTCTCGTC ATGGAAGGTT CCAGCAAATG TGAGATACTT TTATGATTTC  720
ATTTTCTCCA AAAGAAAGGG AATAAGAGAA GAGGGGAGGA AATAAGACTA ATTGCGAGAG  780
ATAAAGTACA AGGGTGAGGG AAGGAATAAG GAGACATGAC GGCAGCGTGG AGCAGCCGAG  840
GGGGGAGATT GCTTTCACCA CTTCCCAGCA TCTATTGCAG ATTCCACCCT CAAACATTTT  900
GTAAGGACTC TTTATTCAAG GTAACGTTTG AACCCTGCTG AGCCAGTGGC ATGGGTCTCT  960
GAGAGAATCA TTAACTTAAT TTGACTATCT GGTTTGTGGA TGCGTTTACT CTCATGTAAG 1020
TCAACAACAT CCTGGGATTG GGACCCACTT TCTGGGCACT GCTGGCCAGT CCCAAAATGG 1080
AACATAAGGA AGTGGTTCTT CTACTTCTTT TATTTCTGAA ATCAGGTAAG ACATAGTTTT 1140
TTTAAATTAT AATAATTATT TTTTCTCCCA CAATGTAGTA AAAATACATA TGCCATGGCT 1200
TTATGTGCAA TTCATTTAAT TTTTGATTCA TGAAACTTCC AGTTGAAAAT CTTGTATAAG 1260
ATTGAGGAAT TC                                                      1272
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FIG. 2A

FIG. 2B

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CCCCAGTGTC TTTAGTTGCC ATCTTTATTT ATGTCCAAAT GCCCGACTGT GTGTTCTTAA   60
CTAAACATTT TGATTCATAG CTACCCATTC TACTTCCAGT AAACAGAAAG TTTTATTTGG  120
TTAATGCTAA CCAAATAGAT TAAAAGGAAG TCATGACAAT TAGACATTGA CATTGATTTA  180
CTGACCATTT ATTCCACTTG GATCTCCCAC CTCTAGGTCA AGGAGAGCCT CTGGATGACT  240
ATGTGAATAC CCAGGGGGCT TCACTGTTCA GTGTCACTAA GAAGCAGCTG GGAGCAGGAA  300
GTATAGAAGA ATGTGCAGCA AAATGTGAGG AGGACGAAGA ATTCACCTGC AGGTATTTCC  360
ATTGTCGTTG CACCTACGCA GGAATCTGTA ATTCAGATGG CAAGTAATTT ACTCACAAAT  420
TTATTAATGA TTTAAGAGGA AAGAGAAATT TATGGAGCCA GAGTTTGGAA CTATATTTGC  480
TCACAGTATG TGAAGCCATA CTAACAGCTT CTTGTTAAGG TTTATTGGAG TCTTTGTTAG  540
AAAAATACCC TCAAAGGAAG TTATTTGTTT TTACACCGGA CACAAACATT AGCAGTTATT  600
GTTCTGAGCT CCAGTTTTCA ACATCATCAT CAGTAAATGT TTGTTGAGGA TCAGGTGAAT  660
GAAAGTGTCC TAGATAGATC TGAGCAATGA CTTATAGCTA CAAGATCCAG TGCCTGCCCT  720
TTAGTATTTA AGGTGTAGTC AAAGAAACTG GATATAATGT TAAAAAAAAA AAAAAGACAG  780
CCCAAGTGAG GTACAGGCAT AATCAATGCA TGCTCTACCC AGATCCAGAA GAAAGAACAG  840
TGCCTAAGGT TGAGGCAGCT AGAGAAGGCT CAGGGAGGAG GTGGGAACTG AGCTGGGTTT  900
GGAGTTGAGA GAGCTCTTGA CAAGCACCAG GAAGGCAGGG GAAGATGCGG CCCTGCACCT  960
TCTGAGGGGG ACCATTAAGA GATGAAGTTG ACTAAAGCAG AGACTTTGTG TAGGTGACGG 1020
GCTTGGGAAG GTAGCTATGG AATCCAGACT GAGCACCCAT AGCAGGACCA CGGGATGGAG 1080
ATGGGAGGGG TCAGGGGCCA GGGTGGGGTG GAATGTGGAG CAGAGGTTCA GGGGAACTGA 1140
TCAGAGTTGG GAGGTCATGG AGACGGACTA TCTTGGCGAA TGGGTTCAAA GCAACCAGAG 1200
TTGCTTCTTT CCAACCCAAA AACAAAAATT AAGAAGATGA GTGAAGAAGA AGTAAAGCAG 1260
TTGAAACAGG AAGAAAGGGA AAATTATGAG GGAGGGAAGG TAAGGGCAGA TAAGATTTGC 1320
TGCCACGTTG GTGTATTTTG TTCAGTACTT CATCGATGCC ATGCCCAAAT AACTGAAAGA 1380
GGCAGCAATT CTGAACTCTC TGGTCCCTCA AGATATTCAA TGATCTTTAG CATGTCTCAC 1440
TTATTAATAA ACATTTGTTT TCTTTAAATA AAGAAAAATA CTTATTGGAT TTCCTGCTTC 1500
GTTCTGCAGG GCATTCCAAT ATCACAGTAA AGAGCAACAA TGTGTGATAA TGGCTGAAAA 1560
CAGGAAGTCC TCCATAATCA TTAGGATGAG AGATGTAGTT TTATTTGAAA AGAAAGGTGA 1620
```

GTACATTTTC TTCCTCCTCC TCCTACTGTC CTCCCCATCC TCCCACTCTT CCTCTTTCTC 1680

TATTCTATCT TTAATTTATA AGACCAGAGG AGGAAGGCAC TATCGTGTTA TAAAACTGAA 1740

TTC 1743

FIG. 2B CONT.

```
CCAAGACCTC TGGCTGCACT GTGCCCCGTG GTGTCCCCAG CATCCTGGTG GGGCTCGATA    60
CACAGAGAGC TCATAAGTAG CATTTGAATA CATGAATCAA AGAATGGCTC AGTTTACTGC   120
AGCCTTTTTG CAGATGCAAA AGATGATCTT TTAGAAAGCA GAAACAGGGG GTCTGGTGCA   180
TGAGATCTTT TTCTCAACGT GACTATGCTG TGCAGACCTT CATGTGGTGT CTTGTGAAAG   240
ACTTTGACCA CTGTGTGGAC TTCCCTTCAG TGTATCTCTC AGAGTGCAAG ACTGGGAATG   300
GAAAGAATTA CAGAGGGACG ATGTCCAAAA CAAAAAATGG CATCACCTGT CAAAAATGGA   360
GTTCCACTTC TCCCCACAGA CCTAGGTAAG ACATTCCCTT TCATCTTTGT GTTCATCTAC   420
TGTAAAGTTG TCCCTCTGTG TCTGTGAGGG ATTGGTTCCA GGACCCCTGT GGCTACCAAA   480
ATCCATGCTT CTCAAGTCCC TTATATAAAA TGGTGCAGTA TTTGCATATA ACCTACATAC   540
CTTCTCTTGT ATAATCCCTA ATATAATGTA AATGCTATTT AATCGTTGTT ATACTGTATT   600
GTTTTTATTT GTATTATGTT TTATTGTCAT ATTGTTATTT TCTGTCATCT TTTTCAAGTC   660
TTTTCCATCC ACAGTTGGTT GAATTTGTGG ATCTGGAACC CCATGGATAC AGAGGGCCAA   720
CTGTATTTAG GATAATTTCA TCACTTTTAA TTCAAACCAC AATATGTGAA TAAGCAGATA   780
GAAAGAATCA AAAAGATGTC GATGTTCAAC TATTTTTGGC ACCATAGTAG AACATGGTTG   840
CTTTCTATTT TTTCTTGGAT ATGGAGGTTT CTTGAAGACC TAGAACATAG AAGAATGCCT   900
AGTTTAAAAA AAATCAATGA AACTATGAGT TTTAGGCCAA ATCTGAGAAA AGATCAAAGA   960
TGACTATGTT TGGGACTGAA GTAAGCATAT CAAGTTAGAA CTCTCATCAC ATGTTCGACT  1020
CAAATTGTGG AGCAAAAGAG TAAATAAGAT ATAAAAATGA AAATGAAGAT ACGTGAAATT  1080
CAAATGTTGC AACTTGCCTA TTATTTATTT TAGTGCATTT TTTTGTACTT TTCCCAGTTT  1140
GGTGTTAGGT GGCATTAAGT TCTCAGTAAT GACGCTTATC AAATAGGAAC TTAGTGCTTG  1200
TTACTCACCT TTATCCATTC CCCCAACACT CAACAAATTG CCTTTGCTAT ATCCCTATGA  1260
GATGAGCAGA TCAAATATTC CCCGTGAGTT AATGAAAACT GATTCAACCA AATGGCAAAG  1320
TCAGAGACTA TCGGGGGCCA TGGAGACACT CTGGGCCATT TTTATGAGGT AGTCTAGGCT  1380
CATCTTTATG AGGGAACTGA GGTCTCGGGG GGTGGGGG                          1418
```

IV

FIG. 2C

```
CCTCATAGCT ATTTACACTT AGGCAAGTTT TGTTTTGTTT TGTTTTACGT TGCCACTCAG    60
TTTTCTCATC TGTAAAATAG GGATAATAAC ACCTTCCTCA AATGGTTTTA TTAGGACTAA   120
AAGAGAGAAT GTGTGGAAAG ATGTTAGTGG AATTCCTGGC AGATAGTTCA CATGGACAAA   180
ATGGTATTAA CTACAAAAAT TTTTACAGAG AAAACGGTAA CTGACAAAAG CAGGTGTTTG   240
GAATGAATTA AGACCATGGC AGCCTTTTGA GGCCTTTATA TTTCTCCTGA CTGTGCAATA   300
AAAATATTTT GGCTCTCTAA GACTTGGCTG TCACAGTAGC AATGGTAATA TTAGCTACTG   360
TGCCAGAAGC AGCCTATCAA TAGAGAAATT GAAAATCTGA CCACACAAAT GCTGCAGCAC   420
CCAGCTGAAA TGCATTTGGA TGACAATCTC AGATGGGAAT CGAGAGCATC TCCTTCTGCC   480
TTGCTAATAG CAAGCTGATT TTTAGAATAT AGTCTAAGTG CTTCTTTTCC ATCCTCCCCA   540
GATTCTCACC TGCTACACAC CCCTCAGAGG GACTGGAGGA GAACTACTGC AGGAATCCAG   600
ACAACGATCC GCAGGGGCCC TGGTGCTATA CTACTGATCC AGAAAAGAGA TATGACTACT   660
GCGACATTCT TGAGTGTGAA GGTCAGGAGT GGTTCTAGAA AATGTTTTCA TTTCTGCCCT   720
TCACCTGTAA AATAATTTGT TGTAAAGCCC CTTCCCACAG GGATGTTATT AATAATTGAG   780
TAACGTATTC ACCTCTCGGA AAGAAGCAAA ACCCCAGAAT TAACCTGAAT TTTTTTTTTT   840
TTCTGAGACA GAGTTTTGCT CTCGTTGCCC AGGCTAGAGT GCAACCGTGC AATCTCGGCT   900
CACCACAACC TCCGCCTCCG GGTTCAAGAG ATTCTGCTAC CTCAGCCTCC CAAGTAGCTG   960
GGATTACAGG CATGTGCCAC CATGCCTGGC TAATTTTATA TTTTTAGTAG AGACAGGGTT  1020
TCTCCACGTA GGTCAGGCTG GTCTTGAACT CTCGACCTCA GGTGATCCGC CTGCCTCAGC  1080
CTCTCAAAGT GCTGGGATTA CAGGCATGAG CACCATGCCC AGCAGACCTG AATTATTTTT  1140
ATTAAAATGT TACATCAACA TGTACAAATA TAAAACTACA TCTAAACTCT AAGTACAAAC  1200
TTCTTATGCT TACAACTCTT ACACAGTG                                     1228
```

FIG. 2D

```
TTTTAAAAGA TCATTATTGA AATGAAGATG CCAAATATTG AAAACTCCTA ATGGAGAACG    60
TAGACTCCTG GGAATATATG CACCCTTGGC TCCCCACTGG CCTGTGCATC CCGGTCTAAG   120
GACATGGCAT CATGGAAATT CTGAACTTGG TCATGACTAC AATAGTTGAG GGAGTATTGA   180
CTAAAATATG TGAATGTTAC GGTTTAAAAG GAAAATGACA TTTGGATTAT GCTAGAAAAT   240
CCTGAGTCCT TATTGCCAAT TTTATTGCCA AGTGCCTGTT GTGAATTACA TCGGAATGAG   300
AGGCAAGTCG CACTTAAGTG AGTAGGATTC TGGTTTTTAC TCTCTATTTT GCTTCATCCA   360
TTTCAGTTTT CTTCTTCCTC TCTGTCCTTC CTTCCCACTC TGTCCAGAGG AATGTATGCA   420
TTGCAGTGGA GAAAACTATG ACGGCAAAAT TTCCAAGACC ATGTCTGGAC TGGAATGCCA   480
                                VI
GGCCTGGGAC TCTCAGAGCC CACACGCTCA TGGATACATT CCTTCCAAGT AAGTCTCACT   540
GGGAAAAACA TTCCATGTTT AATTAAGGCT CTGCAGCTCT ATCAGACATT TGCTGTCATT   600
TAGATATTTT AGCATTCCTC AAGAAGTGAA CGCCTGATGT TTTTAATTTC AAAGCTAACC   660
TCCTCCCACA ATATTGCAAG TGAAATACGC ATTCTTGCTG CTCAAAATAT GGTCCACGGG   720
TCAGCAGCAG GGATGTTTTC TGAGAGTTTG TTAGAAATCC AGAA                    764
```

FIG. 2E

FIG. 2F

```
CCAAAATGAT AAGGTCACTG ATTCTGTTGA GTGATTTTTA CACATGTAAA CTGTTAGAAA   60
AACAGTGCTT GGCAGCCGGG CATGGTGGCA CATGCTGTAG TCCTAGTTAC CTAAAGGGCT  120
GAAGCGGGAG GATTGCTTGA GTGAGTTCAA GGAGTTCAAG GCAAGCCTGG GCAATAAGTG  180
GGACCCTGTC TCTAAAAACA AACAAAAAAA AGAAAGTCCT TGGAATACAG GGCCAACCTT  240
GTTTCCTAGT TGCCATCTCT GAACACAGCC TTCATCTGAT TACCTCCTCC ATGCCCGACT  300
GTGCCTAGCA CACAGCAGGT GCTCAATGTT TGCTCTTGAA AAAGAGTCTT ATCCATGAAT  360
GTAAATGTTC AGTGCTACTA AAATCTTTCT TGTCCATTCA GATTTCCAAA CAAGAACCTG  420
AAGAAGAATT ACTGTCGTAA CCCCGATAGG GAGCTGCGGC CTTGGTGTTT CACCACCGAC  480
                                  VII
CCCAACAAGC GCTGGGAACT TTGCGACATC CCCCGCTGCA GTGAGTATGA TGCACACCCA  540
GATTCCAGGA TTTGGACCTG CCCTGTTCTT GAAATCAAAA GAAAACATGT GTCAGTGCCT  600
GAGTGCAGCC TCTGAAAAGT GACCTACAAG TCCTATGGGA TGTTATTGGT CTTTATTTTA  660
TTGCTGGTTT AAAACAGTTA TGGTTATTGG TTACTGTGGG TGATTGATCA GAGCGTCCAT  720
TTATCATGTT TTTCTTTCTT TGCAACTGAA ACTTCTGCCT CAGGAGTTCA CTGAAATGTA  780
GGCTTTAGGT GTTGTTCATC CTATTCTCTC TGTGCTAAAG GGAAATCAGA CCCATGCTCT  840
CTGACACATG GATTTCATTT TCAACCAGAG TTCTAATAGT TGTTTTGTAA ACAAAGAGTG  900
TCTTTCTTTA CAATGTTCAG GTCTGTGGGT GTCCAGTTTT TCCACCTTGG GGAGCAGAGG  960
GTGAGTGGTG GGGGTGGGGA AGAGTTCAAG AGGAGAAGAT GAAATGGCAG ACCTAGTAGA 1020
AATGATGTGG AGTAAACAAT TTTATCATAT TTTCCTCTCT GAGAATTTGA AGCAAAGGAT 1080
TACACACTAA GAGAAATACA GGCATGAAAG GTTAAAAAGG ATTCAGTGAG GGTTGGCCTC 1140
CCCTCCTTTC CTCTGACATG TGTCCTTTGA AAGCGGAAGT TCCTCAGGCA TTCTCCCTTT 1200
TTATGAATAT TAATTTCTCT TTTTTTTCAG TTTCTCTTTT TGTCATCTTT TTTCCTCAAG 1260
AATATCTTGA TTTCTGGATG CACACACTTT TCCTTGGAGG TGTTTTTTGC CTTCTTTCCA 1320
TGGACTCTTT CCCTGTTGTT TGGCTTTTAT GGCATGTTGG GTGCCATTCA GTCATGTCTA 1380
CTCAGTGAAT AATTTATTCT TCAGGAAAGA GAGTGGACCT TTGGTGTATG TGAGAATTCG 1440
GGGTGTGAGG TGACACGTGT TGATACTTAC CAGGTAGGAA GAACTGAGCA AAGAGAACAT 1500
AGAAAGAAGC ACCTACCCAA GGGTCTTTCT CTGAAGGAGT TCCTTGTGAA AGGGTCTCAC 1560
AGGCATAGAT GCTACTAAAT TGATTTCATC TGAAAACATG AAACAATTCT CAAGTGCCAA 1620
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ATTCCAAGAG AGGCTGAGCA GAAGCCAAGA CAGGCCAGAA CACCCTGCAG CCATCCTCCT 1680

TAACATCCAT CTGTGCATTC TCTATTTTAA AATTATTCAT TGTAGGGCTG GGCACGGTGG 1740

CTCACGCCTG TAATCCCAGC ACTTCCGGAG GCCGAGGTGG GTGGATCACG AGGTCAGGAG 1800

TTCAAGACCA ACCTGGCCAA TATGATGAAA CCCCACCTCT ACTAAAAATA CAAAAAAATT 1860

AGCCAGTTGT GGTGACACGC ACCTGTAGTC TGAGCTACTC GGGAGGCTGA GGCAGGAGAA 1920

TGACTTGAAC CCAGGAGGCA GAGGTTGCAG TGAGCTGAGA TCGTGCCACT GACTCCAGCC 1980

TGGGCGACAG AGCGAGACTC CGTCTCAAAA AATATATATA TTCATTGTAA CTTATTTTGC 2040

CCATTCAAGC AACACCTCCA CCATCTTCTG GTCCCACCTA CCAGTGTCTG AAGGGAACAG 2100

GTGAAAACTA TCGCGGGAAT GTGGCTGTTA CCGTGTCCGG GCACACCTGT CAGCACTGGA 2160

VIII

GTGCACAGAC CCCTCACACA CATAACAGGA CACCAGAAAA CTTTCCCTGC AAGTAAGTCC 2220

CCTCCAGTCT CATTCTGCTG CTATGGAATG TGAAATCCCA TTGACTTTGC CTTAGTTTTA 2280

GTTACTGTAG GAACGCAGGA TAAAGTATTC TGGAAGAAAA ACTGATCTAG TCATAAGTAA 2340

AGGAAATGAA CTTTAGCACG TTTTTTCCCG TAACGGTTGT TCTCAAAGCG TGGTTCCCTA 2400

GACTTTTTTC TTTTTGGAAA GCTAAACTCA CAATCACTTC TTTTTCAGAA ATTTGGATGA 2460

AAACTACTGC CGCAATCCTG ACGGAAAAAG GGCCCCATGG TGCCATACAA CCAACAGCCA 2520

IX

AGTGCGGTGG GAGTACTGTA AGATACCGTC CTGTGACTCC TCCCCAGTAT CCACGGAACA 2580

ATTGGCTCCC ACAGGTAAGC AAGGGTATGG GAGCTTACTG AGGGCCCAAG TTTTCTCCTT 2640

ATTTTTGTAT ACCAGTGGCA TCATCACAAT ATACAGTAGC TTTGTAAGTT TAATGCTATT 2700

GTGGTCAGAA AGCCTGCCCT TATGATTTCA GTTTTTTTAG ATTTGTTGAG GTTTGTTTTA 2760

TGGTTCAGAA TATAGCCATC TTGGTGAATG TTTCATGTGC TCTTGAAAAG AATGTGTCTT 2820

CTGCGGTTGT TGGGTGGGGT GTTCCCTCAA GGTCATTTAG GTGAAGTTGG TTGCTGGTGT 2880

TCTTCTGTAT CCTTACTGAT TGTCTGTCTC CTCCTTCATT GACTACTGTG GATGAATGGT 2940

GATGTGTCCA ACTTTAACTG TAAATTAGTC TATTTCTCTT TTAGATCGTA ACTCTTTTGT 3000

ATATTTTGAA GCTCTTTTGT TAGGCACATA TGTATTTAGG ATGGTTATGT CTTCTAGATG 3060

AAAGGACCCC TTTATCTTTA TGTAATGTTT CTTCTTATCT CTGGGAATAT TTCTTCTTCT 3120

GAAGTTCTGA ACTCTCTTTA TGGTGATATA AATACAGTCT CACAGCTCTA TTTTCACTAG 3180

TATTTGTGTG ATATATCTTT TAAATTTGTA TGATATATCT TTTAAATTTA TCTGAGCTTT 3240

TAAATTGAGA TGTTCAAACC ATTTGCATTC ATGCAATTGT TAATAGAGTT GAATTTACAT 3300

CTACCATCAA GTTAGTTATT TCTCTTTGTC CCATTTAAAC TTTGTTCCTT TTTTCATCTT 3360

FIG.2F CONT.

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TTTCTGCCTT CATTTAGATT GAGTTTATCT CCACTACTCA CTTAGTAAAT TAATTTTTAA 3420
TGGTTTTAGT ATTTTCCACA ATGTTTATAA TATACATTTT TGACTTTTCA CATTCCACCT 3480
TCAAATGATA TCATTCTACT TGACATATGA ATCCTTACAT CATTGCAGTT CTACTTCCTC 3540
CCTCCCAAAA TGCTATACTA TTACTCTTTG TAATAGAAGC TTACTTCTAC TATGTCACAG 3600
ATCTCACAAT ACATTGACAC TATTTTTGCC CTAATAGTTG TGTTTTAAAG TGATCAAGAA 3660
TAAAACTATT TTAAATATTT TCTTTATTTA TTTATTTTAC CATTTCTGGT GCTTCTCATC 3720
TACTGGGGTA GATCTCAATT TCCATCTGGT GTCAGTTTCT TTCTGTGAAA AACAACTTTT 3780
AGCATTTTTT GTAGCACAGG TCTGCTACTG CTGAAGTCTT TCAGATTTTG AGTGTCTGAA 3840
AAAGTATTTT GCCTTCAGTT TTTAAAAGTA ATTTTGCTGA ACGTAGATAC TGGGTTGAGA 3900
GTTTCATTAC TTGCAACACT TTAATGATGA TGTTCCATTA TCTTCTGTTT TAAATAGTTT 3960
GACTAGTAAT CTGATCTTTG TTCCTATGTT TTCAATAGGT CATTTTTCTC TGACTACCTT 4020
TAAGATTTTC TCATCTTTGT TTTTCAACAG TTCGACTATG ATGTGTTTAT TATTAATTTC 4080
TTTGTGTTTA ATCTGCTTGA GGTATTCTGA GTTCCTAGAT TTGTAGATTG TTGATTTTTT 4140
TCTTTTCTCT TTTTTCTTTT CTTTTCTTTT TTTTTTTTTT TTTTTTGAGA TGGAGCCTCA 4200
CTCTGTCACC CAGGCTGGAG TGCAGTGGCG CAATCTCGGC TCACTGCAAA CTCCACCTCC 4260
CAGGTTCAAG TGATTCTCCT GCTTCAGCCT CCTGAGGAGC TGGGACTACA AGCATGTGCC 4320
ACCAGGCCCA GCTAATTTTT GTATTTTTGG TAGAGACAGA GTTTCGCCAT GTTGGCCAGA 4380
CTGGTCTCAA ACTTCTGACC TCAGACGGTC CATCACCTTG GCCTTCCAAA GTGCTGACAG 4440
TACAGGTGTG AGCAACCGTG CCCAGCCTAG ATTGTTGATT TTCATTGTCC TTGTAAAATT 4500
CATAGCCATT ATCTGTTCAA ACGTTTCTTT TTGCACTTTT CTCTCTCTGT ATTTTCCTTT 4560
TGGGACTCTA AGTACCACGT GTTTGGGATT CTAAGTACCC ACAACATTCA TGTTGTTTCA 4620
TAAATCTTGT AAGCTTGTTC TCTTTTTTTT TCAGTAACTC TTTTTCATTC TTTGTGTTGG 4680
TTTGGATAAG TTCTGGTAAC CTATTTCCAA GTTTATGGAT TATTTTTTCA GTTGTTTCTA 4740
GTCATCTCCT CAGCCCATTG AGAGAATTCT TCATCTCTGA TATTATGACT TTTTTTCTAG 4800
CATTTTCATG TTACTCTTTT CTATAGTTTC CATCTTTGCT GAAATTCTCT ACCTATCTAT 4860
GCATACTGTC CACCGTTACA ACAAGATCCT TTAACATACT AATGTAGGTA TCACACAATC 4920
CCAATCTGAT AGTTTCCAGA TGGCGTCTTC TCTAAGTCTG GCTCTCTGGA TTGCTTTATT 4980
ATTCAACAGT GGCTTTTTGT TCCCCCTTGG GTTTTTTGGT GTGTCTTATA ATTCTTTAAT 5040
CAAACACTAG ACATTATAAA TAGAAGAACA GTAGAGGTTA CAGTAAATAT TATTTATACT 5100
```

FIG. 2F CONT.

```
TTGAAATGGA CACCCTTGTC TTGCAAATAT ATATCGTGGA TAATTGAGTC AATGTAGTCA 5160
CTAGTTTAAC TGAATTGGGA TTTGTGATTG CTAGTTTTAC CTTAAGTGCA CCACAGATAT 5220
AAATTCCTCC AGTGATGTGC TGCTGCTATC TTTTACTTAG AGTGGGGCCT GGGGTGCTAA 5280
AGAGTTTTCT CCGTGTTCCT ATCCATTCCC AGATTTCAGC AGTCACTGCA TGCCTGCACT 5340
ACAGAGGAGA TATCTTCATA CACATAATCT AACCCCATTG ACACTCGGCT GTTTCTTGTT 5400
ACTGAATGCT CACTTTTTGG TGGACGTAGG AGAATACTTA TCTCCCTGGT CTACCTCCCT 5460
CTTAGGCCAG TTGAGCACAG CTCGGCTTTG AAAGTAGTGA TTTTTCAGTG TTCTTGTGCC 5520
TCCTTCTGAT GGAACTTGTA CCTGTGGTGG GTTTGGAAAG AAAGAGTAGT AGGCTTCTGC 5580
TTCATTGCAA TGCAGGATGT TGGGCACAAG AGGATTCCCT GTAACTTCTC CAAGGGAATA 5640
AGATTTTTGC CTCCACCACT CTCTGAGAAG CTGTGGATCT TTGCCTGCAG TCCTAGATGC 5700
AGGACCATCA CCTGCCCTAT CACCCAGAAG CTTTGGTCTT TGGCTTTGTT TGAGGAAGGA 5760
GCTAGAGAAA TGTGCAAAGC TTTCATGTCT GCCCCCCACT GACAGCCACT CACCACCCAC 5820
AGCCTGCACT GCCGAATGCA TCCTCCTCTC ATCTGCCCTC GTGTTCTCAT GAACACTCAG 5880
TAGGGACCCA TAAAAAAGAG CTTGCATGTA AGTGCAATTT CCAATTATAA GTACTCTATC 5940
TGTTCTTTCA CACCCAGGTT TTAAATGAAA TATTACTAGG AACTTATTAA TGTTCTAAAA 6000
TGCTATAAAT CTATTTTTAT GTTAATCTGT CTGCTAATAC AGAAAAGAGA ACAGTCATAA 6060
TTCTCAGAGG CTACCGTACT GTTTTTGTCA TAAATTGCTT CATGCTTCTT TTTTTTCAGT 6120
AATTGTTAAG CTTGATTTCT TTTATTTTAA TTTCAGCACC ACCTGAGCTA ACCCCTGTGG 6180
TCCAGGACTG CTACCATGGT GATGGACAGA GCTACCGAGG CACATCCTCC ACCACCACCA 6240
CAGGAAAGAA GTGTCAGTCT TGGTCATCTA TGACACCACA CCGGCACCAG AAGACCCCAG 6300
AAAACTACCC AAATGCGTAT GTCTTTGATT TTTACTGTAA GAGGGGCATC AGCCAACTGA 6360
AATTTCTGTT AAAAGAGCCA TGCTTCATGC TTCAAGCCAA CTTCCTAGGA CCAAATTTCT 6420
CTTAGACCCA GAATGTGTAG AAAAATGTCT CAAGAATCTT GCTTTTGAAG AAAGGGCCTG 6480
CGAGAAGAGA AATTTTAGGC TGGCTATTTT TCCTGAGTAG TTTTATGGAT GCAGGAGGAC 6540
ATCTGGAGGT GATGAGGTCA CATTAATTGA AAGCTCAGGA GTACATATGA GCAAATGCTT 6600
AGAAACAGTA CCATTCCACA ATGCCCACTA AATATCAGTG CAATATTTCT ACCATAGAAA 6660
TCTATCATTT TAACCTCCAA CCCCTGAAAT GAAGGTTGAA TTTGCTATTT TTGTCTTGGG 6720
TCACAAGTAA ATATACTTTA TATATATAAG TATGAATATA TATACACACA TATATATGTA 6780
TACATATGTG TGCATATATA AATACACACA TATATGAGAT ATACAAGTAT ACATATATAG 6840
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FIG. 2F CONT.

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TGTGTATATA TATGTACACA TATATGTGTG TATATATATG TACACATATA TGTGTGTATA 6900
TTAGAATATA TATAACATAA ATATGTATAT ATATATATTC TGACCTGTAT AAACACAGTG 6960
GATCCTGAGC ACCAGTGGCC TGAAAGGATA TGGGTTGCTG GGACATGAAG AACAAAAGCA 7020
GGATACGCAG ATGCTGAACA GCGAAAGAGG CCATTAGATG AACAGAAAAC CAGGTCTAAC 7080
AAGGACAGCT TTTCTTCCAT AAATGAGTAC ACAATATATG GAAAAAACTA TTTTTACATA 7140
TTGGAGAACA GATAAACTGA GATAATTTAG AAAGGGAATC AAATGAGATC AACCCAATAA 7200
CTACCTTGGC TTTGTTCCTG GAGACTTCCT GGGCTGAAGA ACAAGGAGAT GGAGCCCAAG 7260
CCGACCACAG CAGTCTTGCT GAACTGAGGA AGGAGACTGG AGTTGGGATT ACTAAAACAG 7320
CTGAGATTTT CTAGGCTAGG TAATAACATG AAAGGAAACA TTGTGGAGGA AAGCAGCTCC 7380
AGGAATGTCC ATAGAAAAGT CCTCAAGTCT TTGGCTAAAT AGAAAGCTGC ATATGCACAG 7440
GGAGAGGTTC CAGAGAGAAA ATAGGATAAA GAACAGCTAC TGGGGAAAGA AAAACTGCAG 7500
GGGAACAGTG AGCTCAATGG AGATGCCAGA GCTCACATAG CACTGGGGGA TATTTGAGTT 7560
CTGACCAGCC TGAGGAGAGA CCTCGCTGAA CATCTTGGGC ATTCAGTAGT CACCACATAA 7620
AGCCAAACTT TGGGAGTAGG ATTAGTGTAT TCCTATAATA AAGGCCACTC CAGAAACAGC 7680
ATAGTAAAGC TGAAAAGCAA GTCTAAAAAA ATCAACACGA TCTCCAAGTA AATTAACTGA 7740
TTGCCAGAAG AAAATTCAAC CCTTTAGAGG CAAACAACAA AATCAAGTTG CTCAGTTATG 7800
TGGCATCCAC AATGTGTGAC CTAAATTTAT AACTTTACCA GACATACAAA AAGCATTTAC 7860
TGTGATCCAT AACCAGGAGA AAAAGCACTC AAAACAAATA AACCCCAAAA TGAAGAAATT 7920
GGCAAGAAGA TTTGAAATAT ATATATATCA TAATTGTGTT CAAGGATTTA AATAAAACAT 7980
GAACATGGAA GAAACAAATG GATAATATCA AAAAAGAAAA ATTATAAAAT AACCAAATAG 8040
AAATTAAATA ACTAAAAAAG TGCATGTTTA ATGAAAAATG TACTGGCTAC CCTTACCATC 8100
AGGTTAGACA TTACAGAAGA AAAAGTTAAC TAGAAAATAA TTCAATAGAA GTGATACAAA 8160
CTGCAGCACA CACATACAAA GACTGAAAAG ATAAAGAAAC AGAGCCTCAA GAATATCTAT 8220
GAAAATATCA AAAGATTTCA TATATGTGTA AAGCAAGTCA CAAGAGAGGA AAGAGATATT 8280
GGGACAGAAA AAAATACTTG AAGCAACAAG AAAAATCTTA TTAGAAGCCA GAAGAAGAAA 8340
ATATATGTTT ACACAGAAGA ATAGTGGTAA AAATGACTGA TGCCTTCTCG TCAGAAACTA 8400
TGCTGGTCAG AAACAATGAA ATAACACCTT TAAAGTGATA GAAAAAAATA AAAAAGATTA 8460
ACATAGAATG TTATATCCAG CAAAAATATC CCTTGAAAGT GAATGTTATA TAAATACATA 8520
TTCTGCCTCC CCCAAAATAA ATAAAACACT AAGAGAATAT TTCATTACTA GGCTTATATA 8580
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FIG. 2F CONT.

ATAAAAGATG TTCTAGAAAT CTATTTTGGT AGAAGAAAAA TAGTGCCAGA TGGGAACTTT 8640
ATACTAAGTA ATGAAGAACC CTGGAAATGG CAAATGTAAA AGATTCATAT TTAATGCCTT 8700
AATTTCTTTA AAAGATAATT GATGGGAGGC TGAGTCGGGC AGATCATGGG GTCAGGAGTT 8760
TGAGACCAGC CTGACCAACA TGGTGAAACC CCATCTCTAC TAAAAATACA AAAATTAGCT 8820
GGGCATGGTG GCACGTGCCT GTAATCCCAG CAACTCAGGA GGCTGAGGCA GGAGAATCAC 8880
TTGAACCCAG GAGGTGGAGG TTGCAGTGAG CTGAGATCGT GCCATTACGG TCCAGCCTGG 8940
GTGACAGAGC GAGACTCAAA ACAAACAAAC AAACAAACAA ACAAAAAGAT AATAATTTAC 9000
TACTTGAAGC AAAATGATAG CAATGTATTG CTACTTTAAC ATATGTAAAA GTAAAAATTT 9060
CTAAATAATA ATAATCACAT AAATAATGTA GGAAATAAAT GGTAGTATAC TGTTCTAAGT 9120
TTCTTGCATT ATCCATGAAG TTATATAATA CACATGGTTG AAGGTGGTAA GTTAAAGAGG 9180
GTTATTGCAA ATCCTAGAAC AACTGAAAAA ATTTAAACTT AGAGGAATAG ATAATAATAA 9240
GAATGTTCCA TTTATCCAAA AGAAGGAAAG AAAGGAAGAA AAAAGAATGA AGAAGATATG 9300
GCAAAGAGAG AAAATACACA GCATTATGGT ACACTTAAAC TGAACTGAAA ATATATTTAA 9360
TATACTCCTA AGCATATTAA ATATAAAGGG ATTAAACATT GCACAGAAAA GGCAGAGATT 9420
ATTAAGCTGA ATAAAAATCA AAGCCCAATT ATGTTCTTTT TACTATACAT GCTCTTTAAT 9480
TGTAAAGAGC TAGTCCAAAA ACCAAGTGTG GAAAATGACA TATCATGAAA ATAAGAATCA 9540
GAAGAAAGCT GGAGTGGTAA TGTTAATCCC AAAGTAATCT ACAAGAAATA ATACCACGAT 9600
GAAAAAGTTA TTTCTTAAGT AAAAAAAGTT TATTCATCAA GACTTAACAA TGCTAAATGG 9660
GTTGCACCCT CATAAGAGCC CTTCTGATAT ATGAAGCAAA CACTGACAGA ACTGAAGAGA 9720
CAAACAGATA AGCCCACAAT TAGAGTGGGA GATATCCTAA TGTCTCTCTC CGTATGGTTA 9780
TACATCTTCC CAAACAAAAT ATAATAGAAA AAATACACAA AAAAATCAGA AAGAATATAT 9840
ATGTTTTAAA GGAAATTGTC AACCTATTTA ACACTATGCC AAACTGCAGA ATACACATTC 9900
AAGTATGCAT GGAGCATTCC CCAACATATA CCATATGTGT GGGCCTACAG CAAGTCTTAA 9960
TAGATTGAAA AGAATTAAAA TGATACAGAG TCTGTTTTTG 10000

FIG. 2F CONT.

```
AGCAAAACAG AATTAAATGA GATATAAATA ACAAAAAAAT TGGGAAATTA TCAAATATCT   60
GAAAATGAAA CAACACATTT CCAAATACTT CATAAGTCAA AGAAGGAATT TAGAAAAGTT  120
TTGAACTGAA TAATAGTAAA AATACAACAT ATCAAAGTTC GTATGATGCA GCGAATGTTT  180
TTAGGGTTTT ATAACTTTAA ATGCTTTCAG TAGAAAATAG AAACATGTAA AAATCAATGA  240
CTTAAGATGG CATTTCTCAA AGTATGCTCT GGAGAAACCT GAAGTCTCTT GAGATCCCTT  300
CAGAGACAGT CTATGAGGTT AAAACACCTT TAAATTTAAA AAAAAAAAGA TTTTATTTGC  360
TATTTCACTT TTATTTCCTG ATAAGTGTAC AGTGGAGTTT TCCAGAGGCT ACATAATGTT  420
TGATCACATT ATCTCTCTGA TGGCTAATAA AATGTGTGAT TGTCTATTAT GTTTAAAAAC  480
ATTCTCAGTT TTGGATGCAA TAAATATTCA TAGTATATAT TACAAAATGA AAGCTCTTTA  540
GGGTCCCCAA TACTTTTTAA GAGTTAAAGG GTCTTAAGAC CAAAAACTTT GAGAACTGTT  600
GATTTAAGAT AACTTAAACA TCTAGAAAAG GAGAAGCAAA TAAGATCCAA GGTAAGTGGA  660
AGGAAGGAAA GAATGAAAAT CTGTGAAATC CAGTGTATAA GAATATAGAC AAACAATTGA  720
GTAAATCTGT GAAACAGAAA GTTGGTTCTT TTGAAAGATT CATGTAATTG ATAAACCTCT  780
GCCTAAACTG ACGACAAAGG AGGGAGCACC ACCGTCAACA TCAGGAGTAA AAAAAGGGAA  840
GAGTCATTGC TATAGGATCT TTTTGATATT AAAGCTAATA AACAAATATT GAGAGCAACT  900
TTACGTTAAC AAATTCAATA ACCTAGATAA TATGGACTAA TTCCTTAGAA AAAAACAAAT  960
AAGCAAATTG GACACTGAAT AAACTGAATT TCTAACCAAT CTGATATCTA TTAAAGACAA 1020
CATGTGTATA TAATCTTTAA TATGTTAATA TATATTAATA AATCAATAAA CTTCCCACAG 1080
AGAACACTCT AAGTTCAGAT GGCATCATTA GAAATTTTAT TATTTAAAAA AAATCCAATT 1140
CTTCACGATC TGTTACAGAA AATAGAGGAG AAGGGAAATA TTTCTTGACT CAATTTGTGA 1200
GAAAAAAAAA AAACCCTAGT TGTAAAAAAG TAGACAAGGA TATTGTGAGA AACTATAGCA 1260
CATTATGTAT TGTGAACATA AATATAAAAA GATGTAACAA AATTTTAATC ATTAACATGA 1320
TGAATATCCC AAACAAGTGA AGCTTCTCTT CAAGAATGCA AGGCTGGCTT AACATTTACA 1380
AAACAATCCA TGTAATCCAA CATGTTAACA GAATAAAAGT GATAAATCAT ATGATTATGT 1440
CAATAGATGC AGAAGAAAAT GTGACAAAAT TTAACACTTA TCCATGATAA AATGTCTTAG 1500
CAAACTATGA ATAGACTGGA ACTTCTTTAA CTTGATCAAA GGCATCTACA AAAGACCTCC 1560
AGATAACATC AACTTAATGG TGAAAGATTA ATGTTTTCTC TCTAAGATTG GGAATAAGAA 1620
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FIG. 2G

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AAATATGTTT GCTCTCAGTA CTTCTAATCA GCATTTTACT ACATTGGTCA CAACCATTGC 1680
CATAAGACCT GAAAACAAAA CAAAAAGAGA GGAAAAAAAG GAAGGAAAGA AAGAAAGGGC 1740
CTAAAGTTTG GAGAGGAAGA ATTAAAACTG CCTGTATTCA CAGAAAGCTT AATTAACGGA 1800
TGCAGAAAGT CCTAAAGATT AATAATTAAA TTTTGCAAGA TTGGAGAACA CATAAGTATA 1860
TACATGATCA ATATAATAAA AGTAGTTGTA TTTTTATACA CTGCCAATGA TCAACTGGAA 1920
AATAAAAATG TCAGAGCAAT ACCACTGACA ATAGTATCAA AACCACAAGA TATTTAGTGA 1980
TACATTTAAC ACAATATGCA CAAGAATTAT GTACTGCATA CTAAAAAACA TTGTTAAGGA 2040
AGGAATCAAA AGATCTAAAT AAAGATATAT CACGCTTATA TATTAAGAGT CAATATCACT 2100
TCTCACCAAA TTGATCTTTG GATTCAGCCC ATACCCAATT GTTAAGGAAG AAATTACAAG 2160
ATCTAAATAA AGATATATCA TGTTTATATA TTAAAAGAGT CAATATCACT TCTCACCAAA 2220
TTGATCTTTG GATTCAGCCC ATACCCAACC AGAATCTCAG CAGTCGTTTT TTTTAAAAAA 2280
TGTGAAAAAA TGTATATGCT AGAATCACAA GGACAATATT TAAAGAGAAG AAAAAAGTTG 2340
GAGGACTTAC TTACCCAAAG GTAAAGACCT ATAAAGGTAC AGTAAACAAG ATATGTGGTA 2400
TTGGGAAAAA AAAGTATACA GATATAGAAA TGGATGGTCC AGAAACAGAT CCACATATAC 2460
ATGATCAATT TAGTTTCTAG GTAGGTGACA AGGAAATTCA ACAGGGAAAA ACATCTTTTC 2520
CAAAATCATT GTGAAACAAT CGGATATCCA TCTAGAAAAC AAAAATAAAA ACAAATTTTG 2580
ACTTCTACTT TCCATCCCAA ATTAATGTGC AAAAGCTCCT AGATCTAAAT GTAAGAGCTA 2640
AAACTTAAGC TGAAATAAAA CAATTCCAGG AAAATATATA ATATTTTCAC AAACTTGAGG 2700
AAGGCAAAAT TTTTTTCAGG CAGGACCCAG AAAACACTAG CTTTAAAAGA AAATAAATTA 2760
TAATTTGGGC TTTCATAAAA TGAAAATTAT GTTCATCAAA AGTCATTGTT AAGAAATCAG 2820
TAGGTAAGTA ACAGACTGGA ATAAAAATTC TCTCCATCCA TATATCTGAC AAATGGTTTG 2880
TATCTAGAGT ATAAACGTTT CTCCCACTCA CTAATCAGAG GACAAACACC TAATTAAAAT 2940
GGGCAACAGA ATTGAATAGG AAATTTCTCA GGGAACGATG GACAGATGGA CAATAAGCAC 3000
CTGAAAAAAA TGCTCAACAT TTTAGCCATC AAAGATATAA GAATTATAAC CATCACAAGA 3060
TGTCACCAAC ACTTAATTGG CATGGGTATC ATTAAGAAGA CACAACAATA AGTGCTGTCA 3120
CTGATGTGGA GCGAGGATGT GCAGCTCTCG CATACGCTGG TTAAAGTACA GTATGCTGGT 3180
TTTCCATAAA GTTAAATAAC TATGAGTCTA CCCCAAAAAA CTGCAATTCT ATTCCTGAAT 3240
ATTTACCCCA TGGAAATGAA AACAGAAGTC CACAAAGAGA TCTACAAGAA TATTCACAGC 3300
AGCTCTAGTT ATTATAACCC CAAACTGTAA ACAACTACAA GGTCAATCAA TGAGAAAATG 3360
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FIG. 2G CONT.

```
AATCGATAAT TTGTGATCTA TTCATATAAT GGAATATTAT TAAGCAATTA AAATGAAGAA 3420
GTGACTGATC CTCTCAAATA GGATGGATGG AACTCAAAAA TATATTAAGG AAAGGAGGCA 3480
GATACATAAG TGTACATTCT GTATGAGCCC ATTTATATCA GGTTTGAGGA GAGGTAAAAC 3540
TAATCTTTAG TGAAGGAAAC CAATAGTATT TTCCCTCTGG CAGTGGGAAG AGGGTAGCAG 3600
GAATTGAATG AGCAGTGACA CAGGGTGTTT CTAGAGTAAT GGAAGTGTTC TGTATCATAT 3660
GGGAGTGTGG TTTACACAAG TATAGGTGAT CATCAAAACT CACCAAACAA CATTTAAGAT 3720
CTGTGCATTT CACACTATGT AAAAGTATAC CTCAACTGAA GAGAGTGGAA ATCTGTTTCA 3780
AATGCTCAGC CTTTTAACAC ATCCAGTTGC TTAGACTATG AACTTCCTCA AATGGGGTGT 3840
CTGGGCTTGA GATTAGATCA CATGTGTAGA GTCGCTAGAG AGACAATGTT GCATTCCCAT 3900
GGTACATAAT ACATTTCCCG TTTTCTCAGA CAGCCACAGG TCATGAATGT GAGGATTCTG 3960
AGAGGTTGGA GCAACATTCT TGGGAGGCAT GAGGGGGAGC ACATTCTCCA AGATCCCCCC 4020
CAGCCCGGGG TCCTCGCCTG CTTTGACTAT TACTCCGTTG TTTTCGGACT CCTCCGTAGC 4080
TGCCCGACCT CTTCAGATCC CATAGTCTCC CTTTATATCT TGAGTCCCAC TGTTCTTCCA 4140
ACTCATCCCC CATTCCCTCA GACCTGGAGT GCAGTGGCCA GCAGAGGATG GATTGAGAGC 4200
AGGAGAGGAT GTCCTGCCCA GGAACCCATC CTAGAGAAAT GCATCCTGCC TGGGAGCTAG 4260
TTTCCCAGGG TGGCTTTGAT ACGTCTTGCA GAAACAAACC CACTTGACAC ACCTGATACG 4320
GTATTGACAG TAACACTATT TTTCGTGGTT GTTTTTCATA GTAAAAGTAG ATCCCTTTAG 4380
TTACACTGTG AGTACTTAGA GTAAGGTGAC TGGCCTGGGA ATGATACCAT CTTGGATGTC 4440
ATTTTCTCCT TGGAGAAATG TATTTTAGTT CCAATGCACA TTTCACAATA CAGTCCTATA 4500
GAGAGAAATA CAGAGAGCTA GACAGTTAGA GATATACTTT TATGTGCATA AAAATATAAA 4560
ATATGCACTT TAAAATCTGT ACCTGTTATT CCTGAGAAAT GTATTTGGCA GAAGGTGGGA 4620
GGGGGATATT CTGATCCTTT TATTTACATG TTTATGTATG ATCTGAGTTT TTATATGGAG 4680
CATATACTAC TTTTGATTTT TTAAAGAAAA ATTAAAATCT GTCTTTGAAA TGTACACAGT 4740
TGTTTAGAAG TTGAGGACCA TTTTTGTTTG TTACAACATT ATTGTACCTA TAATGGGAAT 4800
ATTTCAAAGC CACTTGTTAA CACTTTGTTA GAACAAAATG TAGAGGGTGC TGGGTGCCCC 4860
TGAATATTCT CCCACCTCTT GTGACCTGTA TTGTTTTGGA ATTTCCAGTG GCCTGACAAT 4920
GAACTACTGC AGGAATCCAG ATGCCGATAA AGGCCCCTGG TGTTTTACCA CAGACCCCAG 4980
CGTCAGGTGG GAGTACTGCA ACCTGAAAAA ATGCTCAGGA ACAGAAGCGA GTGTTGTAGC 5040
ACCTCCGCCT GTTGTCCTGC TTCCAAATGT AGAGACTCCT TCCGAAGAAG GTAAGAAATC 5100
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XI

FIG. 2G CONT.

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TGTGGCTGGA CATCTACACG CTTGGACGCT GGGATGAAAA GCCATGGAAA ATCTCACTGA 5160
TGCAGAAACC TTCCATGCTA CACGAGAAAT CAAGTGTTTT TAGAGGGTCT GCCATGTGGA 5220
AGGAAGCCTC AGTGCACTCT CTCAAGGAGG CAGAGGTGTG ACTTTTGGCA CAACGTGAGT 5280
GGGCTGTGCC TTTAGGACAG GTGCAAACCC TCCAAGGTGC TCAACTTAAC CACTCACCTT 5340
GTTCTAAAAT GGGTTATCTC AGTATCCCAG TCCAAATTCG TATTCTATCA TGCTGCCATA 5400
TGTGTGATTC TTTCCAAGCC AGTAAGCATC TCCAGTAATT TCTTAAGGTA GGCAGCGTTC 5460
ATTGCAGTCT TCAGCATTGC AGTTTCTGAG GAATGTGGCC CCTGATTCTG TCATCCTAGA 5520
GAAACCTGAC ATGACTGTAT TGATTCCATA TCATCCTGGG TCTCTGTGGC TCTTCATAAT 5580
CATCCATTTT TTCCCTGTAC AGACTGTATG TTTGGGAATG GGAAAGGATA CCGAGGCAAG 5640
AGGGCGACCA CTGTTACTGG GACGCCATGC CAGGACTGGG CTGCCCAGGA GCCCCATAGA 5700
                                  XII
CACAGCATTT TCACTCCAGA GACAAATCCA CGGGCGGGTC TGGAAAAAAA TGTAAGCCAC 5760
TTTGATTTGG ACTCTTTGGC CTTTTGCTCA CCAATCTTTG CAAACAGAAT TGGTTCTGTG 5820
TTACAGAAAA TCTGACCTGG ACTGCTCTTT TTTGTAATGG GGGAGAGGGG ACAGAAGAAA 5880
ATATTGGAAA GGCATCAGGG GGCTACGCTA GAATATAATT GGCCTTAGTA TGGAAAGTAC 5940
AAGCAGCACA GGCCAGGAAA CCTCCACACA TGTGAGGGTT CTCAGGCCTC TTCCCTTTAG 6000
TGACATTTCT TTAAAGTTTC CATTATTGGG GACTGTCTCT AGTTTCTAGT GTTTGTATGC 6060
TAGGTTCCAG TAATCAAAGA TGCCCTTTAT GAAATTTAAG TCAGATTTTT CGAGAAAAAA 6120
TTTGGATGGG CCATCAGGTC ACCATGGGAC TTCCCTTAGC CTCATCGATT CTCTGCGATG 6180
GTTTACTTTG GGGCCTATGA ATAGGGAAGA CTGAGATATA GGAAAAACCA AAGTGTCTGT 6240
GTTCCCCCAC TCTCACACCC ATGCAGCATA ACACTTCTCA CACCAGATGT GGGGGGATTT 6300
CTCCTCACAC CCCAAGCGAG TCTCCAGCAG ATACCAGCTG GTGTCCTACA ACGTAACGTC 6360
AGTGCTGACA CTCTATCTGG AGACAGCGTC AGATCCCATA AGTTAAGGCT CAGTCCCACA 6420
AGACCGCCCC ACTGCAGATG CCAATCCCAA GTTCCAGGCG GTGACCTGTA CTTCTGCCCA 6480
ACTGGACAAA AATCTGTTTT TCTACTTGAT TACTTTGCTA GAGTGGCTCA CAGAACTCAG 6540
GGGAACACGT TACTTTTATT TACCCATTTG TTATAAAAGA TATTACAAAG GATCCTGGTG 6600
AACAGCCAGA CAGAAGAGAT GCACGGGGCA AGGCATGTGA GAAGGGGCTC AGAGTTTCCA 6660
TGCCCTCTCC AGTGCACCAG CCCCCGGTAC CCCAAGTGTT CAGCAACCCA GAAGCTCTCC 6720
AAGTGCAGTC TTGCTGGGTT TTTATGGAGG CTTCATTACA GAGGCACAGT TGAATACATC 6780
GTTGGCCATT GGAGACCAGC TCACCTTCAG CTCCTGTTCC CTCCCTGGAA GTTGGACGTG 6840
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FIG.2G CONT.

```
GGGGGCTGAA CAGTTCCAAC CCTGCAATCA CATGGTTGGT TCCTTTGGCA ACCAGCCCCA 6900
TCCTGAGACT ATCCAAGAAC CCACCAAGAG TTGCTTCATT CAAACAAAAG ATGCTCCCTT 6960
CACTCAGGAA CCCCCAAGGG ATTTAGGAGC TCCGTGTCAG GAACTGGGGG GCAGAGACCA 7020
AATATACGTT TCTTATTCTA CCACAGTGTC ATATGAATGG GAGGACAACA CTGCCTTTCT 7080
GTGTCTTGCC CCATAGAGGG CGCACAATGC ATGGAAATAA ATGTTTCTGA ATCAACAGCA 7140
AACAGGCTTC ATCGGGTAGG AGAGCGCTGA GCCCTCCAGG GACAATGCAC ATCAATGATG 7200
TCCCACTGTC CTTTGGTGCT GGGGCTCTAA GGCCTCCACT GGGTCAGGCT CCTGAAGGGA 7260
GACCCATTCT CCAAAGACCC CCGAGGGTCA CCACTCCCTG TCCAGGGGTG TGGCCTCATA 7320
GCTCCTTTTG AACAGGGGCA CAGGAAGGAC GGCTTTAGAG CATTCAAAAA ATAACTTTGC 7380
CAAAATAATA ATAATAATAA TAGAAAGAAA GGAAGAAGAG GCTGAGCATG GTGGCTCACA 7440
CCTGTAATCC CTACACTTTG GGAGGCTGAG ACAAGCAGAT CACCTGAGGT CAGGAGTTCG 7500
AGACTAGCCT GGCCAAAATG GTGAAACCTC ATCTCTACTG AAAATACAAA AAAAAATTAG 7560
CCAGGTGTGG TGGCGTGCAC CTGCAGTTGC AGCTACTCAG GAGGCTGAAG CAGGAGAATC 7620
GCTTGAACCC AGGAGATGGA GGTTGCAGTG AGCTGAGATC ATGCCACTGC ACTCCAGCCT 7680
GGGCGACAAG AGCAAAACTC CACCTCAAAA AAAAAAAAAA AAAAAAAAAA AAAGAAGGAA 7740
GGAAAAAGAA ACACTCCTTT ATGTCTTCTA AGGATAGACA TGAAATGCGT GAGCCTTGGA 7800
ACACCTTCTC CCTCTCCTGC CCCACGTGAG CTGGAGCTTA CATGCCTTCT TGTTTTCAGT 7860
ACTGCCGTAA CCCTGATGGT GATGTAGGTG GTCCCTGGTG CTACACGACA AATCCAAGAA 7920
                                  XIII
AACTTTACGA CTACTGTGAT GTCCCTCAGT GTGGTAGGTT GCCTTCTTTT TGGTAAGGAA 7980
ACTGCTTACT TAATATGGAT TTGCAACAAA AAAGGAAAAG GGCTTCTGAG CAGACTGCTT 8040
CTGGGGAGGA GATAGCTGCC CTCTCCATCA GACCCCACTC TTCATCATGG GCATCTTGAA 8100
TCTGCCCTAC TATTGGCCAC ATTTGTTAGA GGAACACCTG CCCATCGCCC CAGGCACACA 8160
TAAATAAAAT AAATGTAAAA TTCCCAAAGA GCAAGCTTAG AGGTAATCTA GTCAGCCCCA 8220
GGATGGTCCC ACTGAATGCT GCCATGTCTA GCGTGGGATG CATGAAAAAT TTAGAGTCAT 8280
TCGGATGAAA AACTTTCCCT TTCCACAGCT GAGAAGTAAG AAAGAAAATA CAAACAGCAG 8340
GAAACAGGTA AGCATGTAAC GCACATTGTA AACCTCAGAT GGCCATCCTA GGAATTCAAT 8400
GAAAGGTAGT GCAGCTCTTT AGCCCCAGAT GGCCTTTCTT ATAAGTTTAC TACTCACAAG 8460
TCACATTAGT GACATAGCTT AGAGACTGCT TGTTGGGTTC CATCCTCATT GCTCTGAGAC 8520
TCTTGTTGGG AGTATGAGGC TTGGATCAGG GGAAGGGGAG TTGACATTAG TTCTTAAAGA 8580
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FIG. 2G CONT.

```
ATTGGAATAA CAAATCCATG GGTATTTCTG AAAAAAAAAA AAAAAAAAGA AAGGAAGCTA 8640
CTTGGAATTG TCCCATATTT AACATTCTGC TGACCAATCA ATTTGTCCTA GTTACAGAAA 8700
ACCACCCTGG ACTTCTCCTA TGCATAATTT GGTTGCTTGT GGTTGGGTCT GCCATGTGGA 8760
GGGACCTTGA GCTGGGGGAA GGAGCTTGGC CTCCAAGTCC ACTGAAGACC AGCATCCTGA 8820
GATTGCCTGG GAAGGTGGTA CAGGGCAGTG ATGAAGATCA TGGGAGCCAC ACTGCCCAGC 8880
TTCGCATTTG GGCTTCTCCT AGGGACACCA AGAGGGAGGA AGGAGGGGTT AGGATGGTAT 8940
GAAAGATTCT ACTTGGCCAA TATTATTGTA ATGCGGCATT GTGATCTCTG GATTTAGCAT 9000
GAGTTGATAG CTGACTTTTT CTGCAGAAGC ATCTTGGTGG CACCTCTAAC TCAAAGTCCC 9060
TCGATGGAGT CAGTTCCAGT TCTCCACTTC TGGCCCCATC TGGTACACAC CACTGCCTCT 9120
CACTGCCTGG GCTCTCTATC CTTGACAGGC TGCCTTGAAG TTGAGCCCAG ACTGATTTTC 9180
TTGCCTCAGA CCCCACTACC GTGCCTGGGA CTCATGCACC TTTGACTCCC ATGGAAGGGA 9240
AGTGCAGTAG TTTCCCAGGT GCAATTCTGG TGTCCTCACC CACATTGAGG ATGTACAAGA 9300
ATCAGGTTCT TAGAGATTGG AGAAAGAAGG AAGAATGGGA ACAAGATTTT TCCCAAAGGA 9360
CTGTGAGGTC CCCCACCTAA CCTTGATGTG AGACAAGTGA GGTTAACCCC AAGCCTGGTG 9420
AGAAGCGTTC CCATCAGACA CTTGGAAATC CTGAGGACTG TTTCATGCAG AAGGATATGG 9480
TTTATTCAGG TTTGACTCAT GCTTGAGAAA GCTAGAGCCT CTGGTGGTGA ATGATTTTAA 9540
TAACTATTTC CTTTCCACCA ACATATACAG TACAAATAAT AATAAGCAAA AATAAATAGA 9600
AACATTCAGT TTTGTTTTGA ATAGTAGGAG CAGGGTACCA TCATTTCTGT AGTTACTCTT 9660
TTAGTACAAC GATGCATGTC TACTGTATGT AAGGCATACT AGCAGAAATT GAGCTCAGCA 9720
CTAGAGAAGA TGATTGCATT CTATGCCTTG CTTCTTTTTT AAAAAAAAGG CTTCCATAGA 9780
TAGATTCTCA GAACAGCCCA TGGCAAATGT AAAGTTATTT GGAAAACCCA GGTTCCAGAT 9840
TCACTAGAGC ATAGAATCTC TGGTTGGTTG GGAAGGAATT TCCTCTTACA GTTGTTACTA 9900
ATAATTGTAT GAACAATTAT TTAAAATATT AACATTTACA TTTGTGAAGA CCTTGAAGGG 9960
CTGGAGACAA CAGAGAAGCA TTTTTGAATA CCCTCTGCAG                       10000
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FIG. 2G CONT.

```
CCCCTGCACT GTTGTAGGCA TTGGTGGATG GTACCAAAGA TGGGACACTG TCCCTACCTC   60
CAGAGACCCT GTGGGCTGGC TACAGAGAGA AGGCAGGGAG GAGGAAAAGA AGAATAAAGT  120
CATATGTTTA AGTCACCCCC ACGGCCGTTG GTTAGTCATG GGAGGCTCCC CAGAGGAGCT  180
GTCCTGAAGC TGGCTGACAG AAGGCAACAT TTCAACTTAG GACAGTAATC CTTGCTACAT  240
ACAATCACAT ACACACACAC ACACACGTGC ACACACAGAG ACTCACATGG AAAAATAAAC  300
CTTTGTGCCT TTCAGCAGTG ATGACAATTA TGGTTTTCAG TAAACTTTAC ATGGTTTAGA  360
TGGTGATGGT GATGATGATG ATTATGGGAA GGATGGCATC ATGTTCTAAA CATACTGCAT  420
GGAGTCAGAA TAACAATGAC AAATAACCAT TTGTCCCAAT CAAGGTTTTC TCAGAAAATA  480
TCTCATTCTG ATGCTAAACT ATACCAGTCT GTTTGATCAC TTCTCCAACA AAATAATTAC  540
AAAGTGCTTA TATTTTCTTG AAAAGAGAGG GTCCTGTGTT GTCTACTACC ACTTTTGAAA  600
CTTAGAGAAA ATGTTCCAAA AGATGATGAT TTTACTATTT AGTTCGGCCT TTAAGATGTC  660
AAAAACTCAG TGCTTGGAAT TTGTCTCGAA TTACACCACA AAATTGCTAC CTTGTCTCAA  720
ATGGGATTTC TTTCCCACCT TGTGCCACAG↓CGGCCCCTTC ATTTGATTGT GGGAAGCCTC  780
AAGTGGAGCC GAAGAAATGT CCTGGAAGGG TTGTAGGGGG GTGTGTGGCC CACCCACATT  840
                          XIV          ↓
CCTGGCCCTG GCAAGTCAGT CTTAGAACAA GGTAAGAACA GGCCCAGAAA CGATTTATAC  900
TGTCCCTCCA CGTAAGCCCT GCAAAACCCT TCTACATTTA CATAAAATCC ACACAGCTGA  960
GGCATCAGCA CCTGCCTCTA AGTTTTCTGA AGGAGGAAAA AAGCTACAAA AATTAATATA 1020
TGTATATATA CATATATATT TTTATAGGTT CTCTACTGTG AAAATGACAA AAATTGCTGT 1080
CTTTTTCTTG ATCTGGGCAG CTCCATCAAA ATCTGTAGGC ACAGTGATTT GCACCAAGTT 1140
CCAATATTGC TGGAAAATAC TGAAGATGCT CTGAGGATTT CTATGGATAT CCATTGTCTC 1200
ATTGTCAGAT GAAAAGAGGG GGAAGTTTTT AGAAATGTGA CACTTTCTGG GTTGGGAGAG 1260
CAAGGACAAA ATTATCTCCA GTCTATCACA GGCACAGATT CTTTTTCTTT GGACACTTTC 1320
GTGAATCATT GAATTCAATG CAGAGGCTAC TCATCCATTC GCAAACAAAA AAATTCTAGG 1380
TCATGATCCC CATAAATGAA GAGTGATCAG TCCAATCCCA GGGAACCTGG ACATTTTGGG 1440
TATTGTTTCA GTGGAACATG CCTTTCATAA GTTCCATTTT CTTGGGTATC TCTTAGGAAG 1500
CAAGCATAGG AAACAGGCCC ATCCGTCTGC CTGTTTTGCT TCCTCATCTC ACTTCTACAC 1560
GAGGGCGCCT GTGCTCAATT GCTGTTTTCC CCTAAAGAGA CTCTTTTCCA TAAGTTTGTG 1620
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FIG. 2H

```
AAATGCCATC GACAAACCTG ATCGCATTGC ATTTCACTCT GCTGTTGAGT CGATTTTTCT 1680
TTATTTTATC ATTTAGTAAC TCCTTGCTCT ACAGAGCTTT CACCTTCCAC ATATTTCAGA 1740
TTCATTCTTT CCTAAACTAT GTGGTGGTCT ACGTCCTCAC TGACTTATCA ACATGCTACC 1800
ATCATGCACT TCCTATCTCT ATTCCTCTTC ATTAAAATCT GGTTCCAAGT GGCTCACACC 1860
ATTATTCTGA GCTATTACCT GCCTACGCAG TCCTAGAAAG TAAGTGATTC AGGAAACATT 1920
CCCCAAAAGT AAAGTTTCTC AGGTAAGATC AGAAGACTCC CATGAGTCAC TGCTGCTCAG 1980
GATCACATCT GGCTCCTTGA AGAGTGATTC ATCAGACCTT ACATAGATCT TGTCATAAAA 2040
ATGAAAGAGG CCTCGGGGGA AGGTCTTGGG CTGGTGGCTT CTGTTGGAGT CCTGGGCTGT 2100
GGGGTGAAAG CCGTGGCTGT AGAGCTTCAT GCGGAGTTAC TTAGCTTTGC TCTCCTGTGG 2160
ACAGGCCATG CTGTGCCTCC CCCAAGCATC GGAAAAATTG GCATAGATGG GCCCTTCTCA 2220
AAAATCCCAC TCCTGGAGCA CTGGCCAAAA TTACTACCAT CCTGATGCTG GGCTTGCAGT 2280
CCTTTCCTTT GGGAATATGA ACATGGTCAA AATTAAGTGA ACGTGTCTTT CTGGCTTTCT 2340
GTACAATGGA GCAGAACAAA GTATCAATTT AACTAAAATT TGAACTAAAT CCTCTTTCCA 2400
GGTTTGGAAT GCACTTCTGT GGAGGCACCT TGATATCCCC AGAGTGGGTG TTGACTGCTG 2460
                                  XV
CCCACTGCTT GGAGAAGTAT GTTTAGGGGA CAATTGACAT GAAGTCTTGT CTTAAATACT 2520
TTTTCTGTCC TTCTTTTCCT CCTTTCCTCC TTTCCTTTCT CACTCTTCCT CCCTTCCTTC 2580
TCTGGCTGTG ACACTAGGGA CCAGGCCAGG GCAATTGGAT AAGAGAGAAG GGAAGGGTTT 2640
CTAGAAAGAA ACTGCAGAGG AAAGACACAG TACAGATGAT TTTGTGGGCC TGAATAAACT 2700
GCAGAACAGA GCTGTTCACT ACCATAGGCT GTATCAGTCT CTGCCCAAAC AGCCCAAGAA 2760
CATTCCTTAA CTGCCTGTTT CAAGCAAATC ATGAATTTTG CTTCTTGCCA CTCAGAAGTC 2820
ACTAATTCTG AGTGGCCAAG GGTGTCAGGG AGACAGCACC AATTTCATGG CACAGAGGTT 2880
ACCTGAAGGG GCTGGACCAT ATTTTCCTCT TGACATCCTC ATCTTTTCTA GGTCCCCAAG 2940
GCCTTCATCC TACAAGGTCA TCCTGGGTGC ACACCAAGAA GTGAATCTCG AACCGCATGT 3000
                                  XVI
TCAGGAAATA GAAGTGTCTA GGCTGTTCTT GGAGCCCACA CGAAAAGATA TTGCCTTGCT 3060
AAAGCTAAGC AGGTACTCGT TCACCTGTGG TCTTCACCCC ACGCTGGTGA AGATATTTGC 3120
TTTATGTCTG GGTTTTATGG GCCATGGCAC TGCATGGCAG TGGGAGGAAC TGTCTATCAC 3180
ATGAAAGGCT CAAGGGCTTT GGGGACAGCA TCAATCTTCA ACCCTAGCCC TGCCACATGC 3240
TAGCTGTGCT CTTGAGAAAG GCAGCAGGAC TCCGTTTTCT CATGTGGAAA AAGAGTTGAA 3300
ATGAGGTACT CTGTTACTCC TAGAACTCAC TTAATGTTCA CCAGTTCATA CACATTCATG 3360
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FIG. 2H CONT.

```
ATCAGAGAAC GATTCAGTTA TTCCAGGCTG ACAATTCCCC CTTCATCATA ATATGTTTAA 3420
GAGAATCATA TAAGACTATA TTTGTTTCAA AGCACTTTAA AAACCACAAG ATCGAGTTGG 3480
GTGTCTGGTG TGGGTGCCTG TAATCCCAGC TACTTGGGAG GCTGAGGCAG GAGGGTCACT 3540
TGAGTCCCGG AGTTTGAGGC TGCAGTGAGT TATGATCGTG TCACTGCATT CCAGCCTGGG 3600
CGACAGAGTA AGACACTGTA CCAAAAAAAA AAACACCAAA AAAACAAAAA ACAAACAAAA 3660
AAAAAACAAC TTCACAATGT CAAAAAAATC ACAAATACAG TTTATAAATG TAAATTATAT 3720
TATTATTATT GTCTTCTTTG ATTTGATTTT CTCTTTCCTG TTGAAATGTT GTTTCACTAA 3780
GCCTGACAAA GTGAAACATT TGCTTATGTC ACTCATTTAG TGCTGTTTGG AGCCAGATAC 3840
TAGTTGAGTC AGCTAAGAAA CAGCTATTTG TAGGAGAAGC AGGTTTGGGA CAGGTGACAA 3900
GGCACGCAGG GCGCTCGCTG TGCTGGTGGT TCTGGAAGAC AGGGTGTCAG TGTGGACAGG 3960
GATGAGCATG GCCTGGATGA GAAGGCACGG GGCAGGAGCC TGAGCTGCTC TCCTGGGCCT 4020
GGCCACAAGC CCAGGGCAGC TTCTCTGGGT CTGTGAACTG AGGGGTGATG TCCTGGGATG 4080
CTCTGACACT CTAGAAGGAG AGAAGAGCCT TTCCAGCTCA GCCTTTATAA ACAGTAGCTG 4140
ATCTCCCTCC TGCTCCCCAG TGTCCTCCCC GCCATCCCAG CAAATGTGCA AATAGAAGGT 4200
CCCCGTTCCT CATGATCCTC AGAGAGCTGG GGTGTTCTGA TGGCTTGAAC AAGTAATTTG 4260
GAAATTTTGG GTTTTGGAGG AGTTCTCTGA TAGGCTGATA CATTTCGAGT TTAGAGTTCC 4320
CACCCCACAT CCCCACACCC CGAGTCTAGG GCATTTAGTG CTCCACCAGG GAACCTGTAG 4380
AGTGAGGAAG TCTGCATGAC AGGCTGGGCC TTCTGATGAT GCTCAGAAGC AGAAAGTGTG 4440
CCTGCTTCAA AGTTGGTGAC GATGATGTTT CTTGATCAGA ATAGGGCATT TCTTATTTCC 4500
AATCCTTTAT CCTCTTGAAC TTACTAAAGT AGAATCAGGT CTAAAAACCG GAGTTCTAAT 4560
GTTTGAGAGT CCCTGGGACT CTAAAGTATA TGAATGTTCT TTGAAAACAA ATACCATTTT 4620
GTTCAAGCAA AAGGCTTATT TCCAATCCTC TTTCATTTGG TATCAAGTAT TTTACTGGAT 4680
TCTTACAACT ATGGCGTAGT AACATTCACT GAGGAGGAAA TGGAGGATCC AAGGATGGAG 4740
CAAGTTGCTC TGGGCACACA ACACATTTGC AATTTTACAG CCTCTTGGTG GCATCTCAGT 4800
CAGACATTCC ATGCACTGAT CAATGCCCTA TTCGATTAAT GTAAAAGGAC ACACTCAGCA 4860
TGAGATTCCA GTTGTGCACA GAATACTACA TGAGAAGTGC GCCTTTGTCA TCCCTACTTT 4920
CAAAGGTGAA GGCCACCAGC AGTATCTTGC ATGCAACTGA TGCCTTTCAA ATGAAACCTT 4980
ACATCTGCAT AGTCCATAGA CAACCACAGG CAAATGTGAG GGTGAAACTC TGTGTTCTAC 5040
GTTGCTCTGT GTCAGTGAAG CAAGGCAGTG CAGTTCAGAG GGCTCTGGGG CCTCAAGACA 5100
```

FIG. 2H CONT.

GGGATGACTG GTTGTGGGTA CTGCAGCTGC GAGCAGAGCA GTCAAACATA ACTGCTGATG 5160

CTTTTCTTTC AGTCCTGCCG TCATCACTGA CAAAGTAATC CCAGCTTGTC TGCCATCCCC 5220

XVII

AAATTATGTG GTCGCTGACC GGACCGAATG TTTCATCACT GGCTGGGGAG AAACCCAAGG 5280

TGAGATAAAT TCCATTGCCC ACATAACGAA TTGGTTTTGA CCTACAGTCC ATGTGACAAA 5340

ATGATCATTT TGGAGAAAGC TGTGCAAATT CCTATCCATG AATGTGGTCC ACCCCACTCC 5400

TGATTTTGCC TGGGCACCTG TCTATGTCTT AATCAGTCTT CAAGGCACAT GATCAAAGGG 5460

AGGAAAACTG TGTCTTTGAG TCTCTCTCTC TCTCTCTGTT TTCAGAACAT TTTTATTTCA 5520

ATTAATTAAT TTTTAACTTT TATTTTAGGT TCAGGGGTAC ATGTGCAAGT TTCTTGTATA 5580

TGTAAACAGT GGTTTGTCAT GCAGATTATT TTGTCACCTA GGTACTAACC CTAGTACCCA 5640

ATTCTTAGTA TTTCCTGCTC CTCTCCCTCC TCCCACTCTT CTCCCTCAAG TAGGCCCCAG 5700

TGTCTGTTGC TCTCTTCTTT GTGTCCATGA GTTCTCATCA CTTAGCTCCC ACTTATAACT 5760

GTGAACATGT GGTATTTGGT TTTCTGTTCC TGTGTTAGTT TTCTAAGAAT AACGGCCTCC 5820

AGCTCCATTC ATGTTCCTGT AAAAGATATT ACCTCATTCT TTCTTATGGC TAAACAGTAT 5880

TCCATGGTGT ATATGTACCA CATTTTCTTC ATCCAATGTG TCATTGATGG TCATATAGGT 5940

GATTCCATGT CTTTGCTACT GTGAATAGTG CTGCAATGAA CATTCATGTG CATGTGTCTT 6000

TAGGGTAGAA TGATTTATAT TCCTCTAGGT ATATCGCCAG TAGTAGGATT GCTGGGTTGA 6060

AAGTTAGTTC TGCTTTTAGC TCTTTGAGAA TCACCATACT GCTTTCTACA GTGGATGAAC 6120

TAATTTACAG TCCCACCAGC TGTTAGTGTT CTCTTTTCTC TGCAACCTTG CCAGCATCTG 6180

TTATTTTTTG ACTTTTTAGG AAGCCATTCT GGCTGGTGTG AGATGATTTT TCATTGTGGT 6240

TTTGATTTGC ATTTCTCTAA CGATCAGTGA TATTGAGCTT TTTTTCATAT GTTTGTTGGC 6300

CACAGGCATG TCTTCTTTAG AAAAGTGTGT TAGTGTCCCC TGTCCATTTT TTAATGGGGT 6360

TTTTTTTTTC TTGTAAATTT GTTTAAGTTC CTCATAGATG CTGGATATTA GACCTTTTTC 6420

AGGTGCATAG TTTGCAAATA TTTTCTCCTG TTCTCTAGGT TTTCCCTTTA CTCCCTTGAG 6480

AGTTTCTTTT TCTGTCCAGA AGCTCTTAAG TTTAATTAGA TCCCATTTGT CAATTTTTGC 6540

CTTTGTTGAG ATTGCTTTTG GCATCTTCAT GAAATTTTTG CCCGTTCCTA TGTCCAGGAT 6600

GGTGTTACCT AGGTTGTCTT CCAGGATTTT TGTACTTTTG GATTTTACAT TTAAGTCTTT 6660

AATCCATCTT GAGTTGATTT CTGTATATGG TGTAAGGAAA GGGGTCCAGT TTCCATCTTC 6720

TACATATGGC TAGCCAGTTA CCCCAGCACC ATTTATTGAA TAGGGAGTTA TTTTCCCATT 6780

GGCTTGTTTT TGTCAGCTTT GTTAAAAATC AGATGTCTGT AGGTGTGTGG CCTTATTTCT 6840

FIG. 2H CONT.

GGGCTCTCTA TTCTGTTCCA CTGGTCTACG TGTCTTTTTT TTTTTTTTTT TACCAGTACC 6900
ATGCTGTTTT TGTTACTGTA GCCCTGAAGT ATAGTTTGAA GCCAGGTAAT GTGATGTCTC 6960
CAGCTTTGTT CTTTTTGTTT AGGATTGCCT TGGCTATTCT GGCTCCTTTT TGGTTATATA 7020
TAAATTTTTG AAGTAGTTTT TTAATAGTGC TGTGAAGAAT ATCATTGGCA GTTTGATAGG 7080
AATAGCAATG AATCTGTAAA TTACTTTGGG CAGTATGGCC ATTTTAATGA TATTGATTCT 7140
TCCAATCCAT GAGCATGGGA TGTTTTTCCA TTCATTTGTG TCATCTCTGA TTTCTTTGAG 7200
CAGTGTTTTG TAATTCTTAT TGTAGAGATC TTTACCTCTC TGGTTAGCTG TATTCTTACA 7260
TATTTTATTC TTTTTGTGGC ATTTGTGAAT GGGACTGTGT TCCTGATTTG CCTCTGGGCT 7320
TGGCTGTTGT TGGTGTAAAG GGATGCTAGT GATTTTTGTA CATTGATTTT ATATCCTGAA 7380
ACTTTGCTGG AGTTGATTAT CAGCTGAAGG AGCTTTTGGG CTGAGACTAT GGGGTTTTCT 7440
AGACATAGAG TCATGTCATC TGCCAACAGG GATCGTTTGA TTTCCTCTCT TCCTATCTGG 7500
ATGCCCTTTA TTTCTTTCTC TTGCCTGATT GCTCTGACCA GGGCTTCCAA TACTATGTTG 7560
AATAGGAGTG GTGAAAGAGG GCATCCTTAT CTTGTGCCAG TTTTCAAGGG GAATGCTTCC 7620
AGCTTTTGCC CATTTAGTAT GATGTTGGCT GTGGACTTGT CATAGCTGTC TCTTATTATT 7680
TTGAGATATA TTCCTTCAGT ACCTAGTTTA TTGAGAGTTT TCAATATAAA GGATGGTAAA 7740
TTTTATCAAA ATCCTTTTCT GCATCTATTG AGATAATCAT GTGGGTTTTC TCTTTAGTTA 7800
TATTTATGTG ATGAATCACA TTTATTGATT TATGTATGTT GAACCAAGCT TACATTCTGG 7860
GGATAAAGCC TACTTGATCA CGATGGATTG GCTTTTTTAT GTGCTGCTGG ATTTGGTTTG 7920
CAAGTATTTT GTAAAGGATT TTTGCATCAG TGTTCATCAA GGATATTGGC CTGAAGTTTT 7980
TTGTTGTTTT TGTGTCTCTG CCAGGTTTTG GTATCAGGAT GATGCTGACC TCATAGAATG 8040
AATTGGAGAG GAGACCCTCC TCCTCAGTTT TTTTGAACGG TTTCAGTAGG AATGGTCATA 8100
GCTCTTCTTT GTACATCTGG TGGAATTCAG CTGTGAATCT ATCTGGTCCT GGGCTTTTGT 8160
TGGTTAGTAG GCTATTTATT ACTGATTCAA TTTTGGAGCT CATTATTGTT CTGTTCAGGG 8220
AATCAATTTC TTCCTGGTTC AGTCTTGGGA GGGTGTATGT GTCCAGGAAT TTATCCATCT 8280
CTTTTAGGTT TTCTAGTTTG TGTGCATGGA GCTGTTTGTA GTAGTTTCTG ATGGTTATTT 8340
TTATTTTTGT GGCATCAGTG CTAACATCCC CTTTGTCATT TCTAATTGTG TTTATTTTGG 8400
TCTTATCTTC CTTTTCTTCA TTAGCCTAGC TAGCAGCCTA CCTATCTTAT TACTGTTTTC 8460
AAAAAACCAA CTACTGGACT TGTTGATCTT TTGAATGAAT TTTCATGTCT TGACTTTCTT 8520
CAGTTCAGCT CTGATTTTGG TTATTTCTTG CCATCTGCTA GCTTTGGGGT TGATTTGCTC 8580

FIG. 2H CONT.

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TTGTTTCTCT AATTTTTTCC ATTGTGATGT TAGGTTCTTA ATTTGAGATC TTTCTTCTTG 8640
ATGCTAGCAT TTGGTGCTAT GAATTTCTCT CTTAACACTA CCTTAGCTCT GTCCAAGAGA 8700
TTCTGGTATG TTGTATCTTT ATTCTCATTA GTTCAAAGAA CTTCCTGATT TCTGCCATAA 8760
TTTCATTATT CACCCAAAAG TCATTCAGGA GCATGTTGTT TGATTTCCAT GTAATTGTAC 8820
GGTTTTGAGT TATTTTCTTA GTCTTGACTG GTATTTCATT GTGCTGTGGT CTGAGAGTGT 8880
GTTTGGTATG ATTTTGGTTC TTTGGCACTT GCTGAAGATT GTTTTATGTC CAATTATGTG 8940
GTTGATTTTT AGAGTATGTG CCACATGGTG ATGAAAATGT ACATTCAGTT GTTTTGGGAA 9000
AGAGAGTTGT GTAGAGGTCT ATCAGATCCA TTTGGTCCAA TGCTGAGTTC AGGTCCTGAA 9060
TATCTTTGTT AATTTTGTGC CTCGATGATC TGTCTAATAC TGTCAGTGGA GTACTGAAGT 9120
CTCCCACTAT TATTTTGTGG GCGTCTAAGT CTCTTTGTAG GTCTCTAAGA ACTTTATGAA 9180
GCTGGGTGCT CTTGTGTTGG GTTCACATGT ATTTAGGATA GTAGATCTTC TTTTTGAATT 9240
GAACCCTTTA CCCCTTTACC GTTATGTAAT GCCCTTCTTT GTCTTTTTTG GTCTTTGTTG 9300
GTTTAAAGTC TGTTTTGTCT GAAATTAGGA TGGCAACCCT TGCTTTTTTG TCTGATTTCC 9360
ATTTGCTTGG TAGGTTCTCC TCCATCCCTT TATTCTGAGC CTATGGGTGT CATTACATGT 9420
GAGATGGGTC TCTTGAAGGT AGCATACCAG TGGGTCTTGC TTTTTATCCA GCTTGCCACT 9480
CTGTGCCTCT TAAGTTGGGC ATTTAGCCCA TTTACATTCA AGGTTAGTAT TGCTATGTGT 9540
GAATTTGATG CCCTCATTGT GTTGTTATGC TGGCTTGTTT GTGTGATGGT TTTATAGTGT 9600
CATTGGTCTG CGTATTTAAG TATATTTTTG TATTGGCTGG TAGCCATCTT GCTATAGTTA 9660
GTGCTTCTTT CAAGATCTCT TGTAAGGCAG TTCTGGTGGT AACCAACTCC CTCAACATTT 9720
GCTTAGCTGA AAATGATCTT ATTTCTCTGT TGCTTAGGAA GCTTAGTTTG GCTGGATATG 9780
AAATTCTTGG GTGGATATTT TTTAAGAATA TTGAATATAG GCCCCAATAT CTTCTAGCTT 9840
GTACGGGTTC AGTTGAGAGG TATGCTGTTA GATTGATGGG GTTCCCTTTG TAGACGACCT 9900
GTCCTTTCTC TCTAGCTGCC TTTAACATTC TGTCTTTCAT TTTGACCTTG GAAAATCTGA 9960
TGATTATGTG TCTTGAGGAT GATCTTCTTG TATAGAATCT                       10000
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FIG. 2H CONT

```
CACAGGGGTT CTCTGTATTT TCTAAATTTG ACTATTGGCC TCTCTAGCAA GGTTGAAGAA   60
GTTTTCATGG ACAATATCCT GAAATGTTTT CTAAATTGTT TACTTTCTCC CCATCCCTTT  120
CAGAAATGCC AGTGATTTGT AGATTTGGCC TTTTTACATA ATCCCATGTT TCTTGGAGGC  180
TTTGTTCATT CCTTTTCATT CTTTTTTCTT AATTTTTGTC AACTGTCTTA TTTCAGAAAG  240
CCAGTCTTCC ATTTCTGAGA TTCTTTCCTC AGCTTGGTTT ATTTTGCTAT TAATACTTGG  300
ATTGCTTTGT GAAATTCTTA CAGTTTGTTT CTCAGCTCTC AGCTCTGTCA GATCCATTAG  360
GTTCTTTTTT AAACCAGTGA TTTTGTCTTT CAGCTTCTAT ATCATTTTAT TGTGATCCTC  420
AATTTCCTTG GATTGGATTT TGCCATCCTC CTGGATCTTG ATGATCTTCA TTCCTATCCA  480
TAGTCTGAAT TCCAGTTCTA TCATTTCAGC CAGCTCAGCC TTGTTAAGAA CCCTTGTTAG  540
AGAACTAGTG TGGTTGTTTG GAGGACATAT GGCACTCCGG CCTTTATGTT CCTTTAACTG  600
CAGTGTAGGT TGAATACAGC CAATAGACTT GTTCTTTGGA TGTTTTTACA GGGCCAAAGC  660
CTTGTGCAGG GTCTTTATTT GTAGTTGATT TCTTGTCTTT GGTTTCATAG TGTGGTATGT  720
TAGCAAGGTA TTTTTGGTGT TGAAGCTTTG GGGTGTGATC CATTTTTTAT TTGTATATTT  780
CCCTACACCT AAAACAAGCA AAAAAACAGT AAAGGTCTTT GAGTCTCTTA ATCCATAATT  840
TCAGCATTCC TGAGTATGCT TCCCTGGGTA AGTGGGGTTT TCACCCAGCC CTCAAGTTAA  900
GAGTGTTAGA TTATTTTTCA TGTGAAATTA GCCAGACTGG CTTTCTTAAC ACAATGTAAA  960
ACAATAACAA CAAAAGTTAT AATTAGACTA GTCTTCTTCC CAAATACCCA CATGTCTAAT 1020
GTAAGTGGGA TGGTGTTAAA CAGGGGACCT ACAACTGGGG GAGAGGCGGA CAGGTCCCAT 1080
GGCCCCAGGT CTAGGATGGC ATTTGGTATT GGTTGATGGG TGTGGATGTG AACAAGAGAG 1140
GGAACACTTG TGCAGGATAT GGTATCAGCA CCTGTAATAC ATTTTAGGGA TTCTTTCTTC 1200
TCTTTGCAGT ATGCCCTGAC AATAATTATA TCCATCAGCC TAGTCCCCTT GGCCATTGAA 1260
ACACTAAGAC TGTCTTAGGA TCCCTGCTGC AGTTTCTCAG AGGTGCTAGG AGGGCATTAG 1320
GAGTCTGAAG CCCTGGAAGT GTGTTCTGAC TTTGCCACTA GCTAGATAGA CCTGGACTAG 1380
GCACGTTACC TCTTTGTACC ACTCAGCTCT AACCCCTCAT TCAAAAACCC AGCATTTTCA 1440
AGTGGTGTTT TTCACATCAG CCTTTGCATA AGTTTTCATT TGAAGAAAGG TTTTTTTGTT 1500
TTTGTTTTCT TGGTTTAATC AAACATTTAA AAACGAATGG TCTAGATGAT TTCAAAGTGG 1560
CTTTCCTTTT CCTGTGCTTT TCCTACTATT TAAAAACTTC ACCTCCTTGA TTTCTTGATC 1620
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FIG. 2I

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TCCCTTTCTG CACTGCTGGG TCTGGGAGCA TTGAGGCCAA GTAAAAGGAA CCTTGGCAAA 1680
GGAGGAACAC CTATGGGTGT GCCAGGCTGC TCCCAGTGTT TTGCATTTTT AAAAATTTAA 1740
ATGCTGCAAA CCTCTATGAA TTACATATTA TTGTTCCTAG TTTACAAATT AGGAGCCTGA 1800
GGCTCAGAGA ATGTGTGGGA TGGTACAGAC TAACCTGAAT TAGAACCCTG GCTCCCATTT 1860
ACTGGCTGTC AGGACTTAGA AAAGTCATAA ACTCTCTGGC TGGGTGCAGT GGCTCACGCC 1920
TGTAATCCCA GCACTTTGGG AGGCCGAGGC AGGCAGACCA CGAGGTCAGG AGCTTGAGAC 1980
GAGCCTGACC AACACGGTGA AACCCCGTCT CTACTAAAAA TACAAAAATT AGCCGGGTGT 2040
GGTAGCACAC CCCTGTAATC CCAGCTACTC AGGAGGCTGA GGCAGGAGAA TCGCTTCAAC 2100
CTGGGAGGTG GAGGTTGCAG TGAGCCAAGA TTGTGCCCAC TGCACTCCAG CCTGGGTGAC 2160
AGAGTGAGAC TCTATGTGAG AAAGAAAGAA AGAAGGAAAG AAGGAAAGAA GGAAGAAAAG 2220
AAAGAGAAAG AAAGAAAGAA AGAAAGAAAG AAANNNNNNN NNNNNGAAAG AAAGGGAAAG 2280
AAAGAGAACG AAAGAAAGAA GGGAGGGAGG GAGGGAGGGA GGGAGGGAGG GAGGGAGGAA 2340
GGGTGGGTGG GTTGTGAACT CTTGTTGATT GTTTCCTCAG CTGAAATGTG GGCTGCAGGG 2400
CTATTGGGGG AGAAACAATA AGAAAGTGCA CCAAGCACCA AGCACATGCT AAGAAGTCCA 2460
TCATGGCAGC TCCTGATAAT AATATGGAAT AGAGTTGTAT CTAACATGAC TCTTTCTTGC 2520
AAGTGACAGA AAATGCAACT TAAGTTGGAT TAAGCAAAAA AGAGAAATCA TTAGTGAACT 2580
GAAAATTCTG CAGGCTCACA TCATGGCCCC AGACCCTGTC CATTATTCTT GGGCACAAAT 2640
GTGACATTCT CGTGGCTGCA GATGCTGTGG TGGCTCTGGC TCTGCAGGAA AAGAAATAAG 2700
GAAGGCCACT CTCCCCATTA CACAAACAAC AGTCTTCCAG CTCTGAGAGG TCGAACTTGT 2760
GTCACCAGCC TGCCCCTAAA CCCGTCACTG ATTAACTCCA ACCTGCATCA GCTGTTCCAT 2820
GCTGGAGGTG GACGCAGGAC CACACTCATA CCAAGATGGG GGCAAAGTGT AGTTCCCTCA 2880
ACAGGATTAT AGGATATAGT GTGATAGGCT GCTGGGCCAG AAAAGCAAAC AGATCCTCTA 2940
CAATTCCTCA ACTGATGAAA GCACGAAGCT AAAATCATAA AGATCTGTGT GTGAGTTCTG 3000
GCTCTCCCAT CTTCCTTGTG AGATTGAGCA GTTAGTTAAT CTCTTTTAGC CTCAGCTTTC 3060
TCACCTGTAC CAACATATAA GGTCATTGTG AGGATTAAGA TTATGCCTCA TGATCATCAT 3120
TATCATCATC ACCATCCACA TTGCAACCAC AACTACCATC ATCATCCCCA CCAACATCAT 3180
CACCACCACC ACCATCACAA TTATCATTAC CACCACCACC ATTGTCACCC TCAACATCAC 3240
CATCATCACT ATCACCACCA CCATCATCAT CACTACCACT ACCAACACCA TCACTCTCAT 3300
CATTCCACCA CCATCACCAT TAACATTACC ATCACTATCA TCACCACCAC CACCACCACC 3360
```

FIG. 2I CONT.

```
ACCCCCATCA TTACTGCCAT CAACATCACC ATCACCATCA TCACCACCAT CACCATCATT 3420
ATCAACCATC ATCACCACCA TTCCACCACC ATCACCATTA TCATCACTAC CATTATTCCA 3480
CCACCATCAT CATCCACCAC CACTACCACC ACCATCACCA CCATCATCAC CATAACCATC 3540
ATCACCACTA TCAACATGAT AGTAATTATG ATTACCACCA CCATTAGCAT TATCATTACC 3600
ACCACCAGTA CCATCACCAT CACCACCGCC ACCACCTCCA TGATCATTAC TACCCACCAC 3660
CATCACCGTC ACCATCATTT CACTACCAGC ACAATTATCA TTACCACCAC CATCACTACC 3720
ACCCTTATCA CAACCCTCAT CATCACCACC ATTCACCAGT GCACCACCAC CACCACCATC 3780
ACTATCATTA ACAATAGACA TCACATAACC AGTTTGTAGC TGGACCTTGA GCCCAGAGCC 3840
CACTCACTGT TTCTTCAGTC CCACCGCCAA CCACCAGGAT GAGTCACAAA ACATAACTCA 3900
GGCCTGCTCC TCAATTTTCT ACATGTCAAT AATGACATTG AAGCAATGGG TGTTCTCTGC 3960
TTCTCAGAGG GAAGTTGAAA TTCTCCTGCT CTTCCCTTCA TGTTTCCAGA TGTTCCCTGA 4020
CTTGGATATT CCAAACGCAG AGTTTGGAGG TGTTGAGGCC AAGGGGTTTT TCCAGGTCAG 4080
CCATCATCTG CAATCACTGA GCTGATCCTG CTGCTGGACT TTCCCTGTTG CCCTCTCCCC 4140
AACGCCCATC GGGGAGGGCT TCAATCCTCA GGTCACCTGT GGCCTTTCTG CCCTCAGAGG 4200
TGCCATCTCT ACATCTACCA CTGGAAGGCA GCACCTACTC ACAGATTGCA TCAATTTCCC 4260
AGCAACTCAT GGTGGGTTTT CCCCCTTATC AGCGTGTTTG CCTTGCTCAG AGAGCAGATC 4320
CCAGAGCAGT GACACCTAAC TTAATTTTCA GCAAAACATT TTGAGAAGGG TGCTCCCTCA 4380
CACAACTACA CAGTCCAGGT GATGCACCCA CTGCCCAATG CTTGGTAGTC AAGAGGAGCT 4440
TCCTCCCTGC AGCTCTGCCC AGATAGGGCT GAG                              4473
```

FIG. 2I CONT.

```
ATTGGGAGCT GCCTCGTGTT CTGCAGCCTC ACAGACAGGA GGTCCAGTGC CGCTGCTCTG   60
TTCTGGAATA TCCTCCTGAA TGTGTTTTGG GTGCAGTTGC CGTTTCTTTC ATCTTTTTAA  120
ACACAGGTAC TTTTGGAGCT GGCCTTCTCA AGGAAGCCCA GCTCCCTGTG ATTGAGAATA  180
                                  XVIII
AAGTGTGCAA TCGCTATGAG TTTCTGAATG GAAGAGTCCA ATCCACCGAA CTCTGTGCTG  240
GGCATTTGGC CGGAGGCACT GACAGTTGCC AGGTAAGCAA AGATCAAGAG ACCAAAGTTA  300
GTCTTGTGCT CTCTTGTCTC AGTCTCAGCC CCTCAGACTT CATTCCCCAG GTGGCAAATT  360
CAAGGATTTT CAACCGAAGA CCCCAGTCTA AGTGTTGTTT AGAAACTTCC TAGATCTGTC  420
CCTGAATGCG TATTCAGATC ATCTAAGGGG ATGTCTTGGG GCTTGAGTTC CAAATCAGTA  480
GCAAGCGAGT TTTAAGTGCC ATAACTACCT CAGGCCACTC ACCCTCCTGG GGTGTGCTGG  540
TGGCCAGGGA CTAAAGTGGT GACTTTTCCG GTAGGGAAGG AGGTAGAGGG TACAGGACAG  600
AGACCAACTG CACACACTTT ACACTGATGC CCAGGCTAGC CCAGTCTAAA GGAAACACCA  660
ACATAGGAAG GGATGTGTGC AGGATTCACA AAAGATCTTT TCTACCCCCC GGAAAAACTA  720
AGTGGTGTGG TTTCGCTAAA CAGATTTTGC TAAGTACTTA AGCACTGCAG ATGCTTGAGT  780
AATATGCTCA TAAGTTCCTT TCTGATTTCA ATTACTGGGA AAATGTATAT ATGGATAGTA  840
GAAGGATGGC ATCCCATAAT AAAAGGCAGG CAGCCTAACC CTCACATGCA TTTTTCTCTC  900
CCTCTGTATA GGGTGACAGT GGAGGGCCTC TGGTTTGCTT CGAGAAGGAC AAATACATTT  960
TACAAGGAGT CACTTCTTGG GGTCTTGGCT GTGCACGCCC CAATAAGCCT GGTGTCTATG 1020
                                  XIX
TTCGTGTTTC AAGGTTTGTT ACTTGGATTG AGGGAGTGAT GAGAAATAAT TAATTGGACG 1080
GGAGACAGAG TGACGCACTG ACTCACCTAG AGGCTGGGAC GTGGGTAGGG ATTTAGCATG 1140
CTGGAAATAA CTGGCAGTAA TCAAACGAAG ACACTGTCCC CAGCTACCAG CTACGCCAAA 1200
CCTCGGCATT TTTTGTGTTA TTTTCTGACT GCTGGATTCT GTAGTAAGGT GACATAGCTA 1260
TGACATTTGT TAAAAATAAA CTCTGTACTT AACTTTGATT TGAGTAAATT TTGGTTTTGG 1320
TCTTCAACAT TTTCATGCTC TTTGTTCACC CCACCAATTT TAAATGGGCA GATGGGGGGA 1380
TTTAGCTGCT TTTGATAAGG AACAGCTGCA CAAAGGACTG AGCAGGCTGC AAGGTCACAG 1440
AGGGGAGAGC CAAGAAGTTG TCCACGCATT TACCTCATCA GCTAACGAGG GCTTGACATG 1500
CATTTTTACT GTCTTTATTC CTGACACTGA GATGAATGTT TTCAAAGCTG CAACATGCAT 1560
GGGGAGTCAT GCGAACCGAT TCTGTTATTG GGAATGAAAT CTGTCACCGA CTGCTTGACT 1620
```

FIGURE 2j

FIG. 2J

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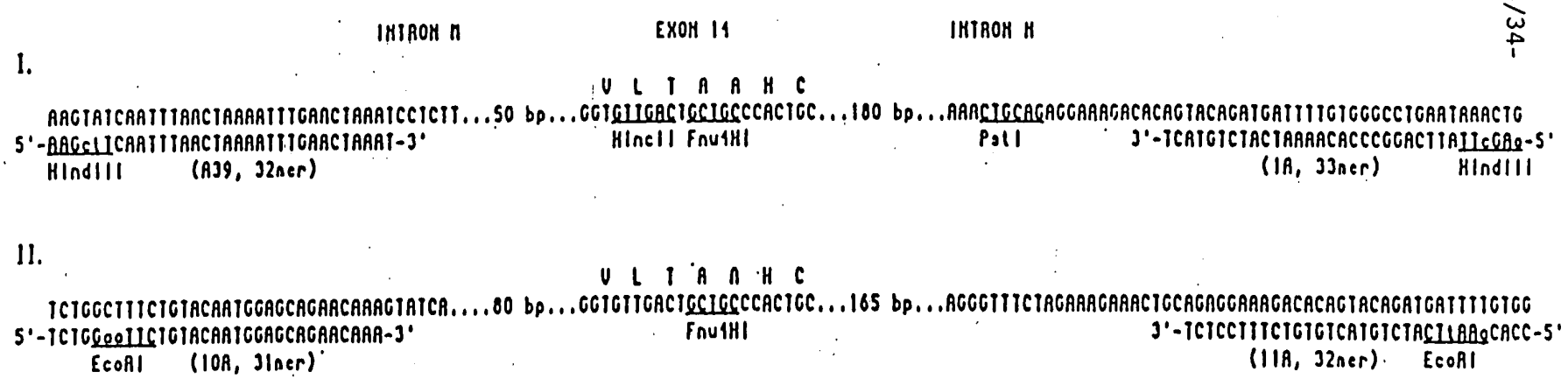
TGAGCCCAGG GGACACAGAG CAGAGAGCTG TATATGATGG AGTGAACCGG TCCATGGATG 1680
TGTAACACAA GACCAACTGA GAGTCTGAAT GTTATCCTGG GGCACACGTG AGTCTAGGAT 1740
TGGTGCCAAG AGCATGTAAA TGAACAACAA GCAAATATTG AAGGTGGACC ACTTATTTCC 1800
CATTGCTAAT TGCCTGCCCG GTTTTGAAAC AGTCTGCAGT ACACACGGTG ACAGGAGAAT 1860
GACCTGTGGG AGAGATACAT GTTTAGAAGG AAGAGAAAGG ACAAAGGCAC ACGTTTTACC 1920
ATTTAAAATA TTGTTACCAA ACAAAAATAT CCATTCAAAA TACAATTTAA CAATGCAACA 1980
GTCATCTTAC AGCAGAGAAA TGCAGAGAAA AGCAAAACTG CAAGTGACTG TGAATAAAGG 2040
GTGAATGTAG TCTCAAATCC TCAAAGAGCT GTGTTTATTT CATTGACAAA TAGATTATTT 2100
GTATCAA                                                          2107
```

FIG. 2J CONT.

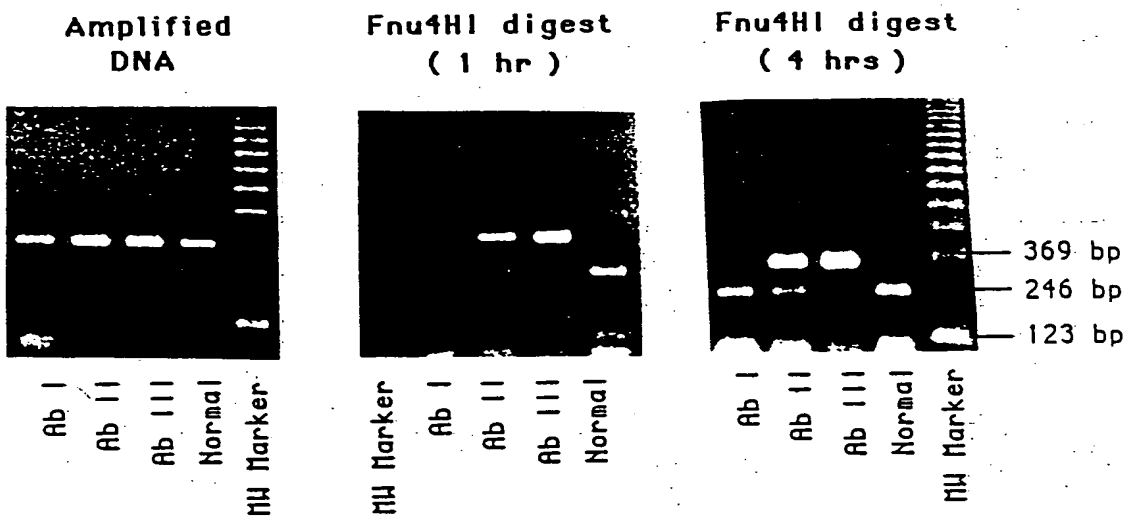
FIG.3



SUBSTITUTE SHEET

FIG. 4

Restriction Digest of the Amplified DNA



EMPLOYEE'S PROPRIETARY INFORMATION AND INVENTIONS AGREEMENT

Gentlemen:

I recognize that Vical, Incorporated (hereinafter referred to as the Company), is engaged in a continuous program of research, development and production with the respect to its business, present and future.

I understand that:

A) As part of my employment by the Company, I am expected to make new contributions and inventions of value to the Company.

B) My employment creates a relationship of confidence and trust between me and the Company with respect to any information which is applicable to the business of:

(1) the Company; or

(2) any client, or customer, of the Company;

which may be made known to me by the Company, or by any client or customer of the Company, or learned by me during the period of my employment.

C) The Company possesses, and will continue to possess, information that has been created, discovered or developed, or has otherwise become known to the Company (including, and without limitation to, information created, discovered, developed or made known by or to me during the period of, or arising out of, my employment by the Company), and/or in which property rights have been assigned or otherwise conveyed to the Company, which information has commercial value in the business in which the Company is engaged. All of the aforementioned information is hereinafter called "Proprietary Information". By way of illustration, but not limitation, Proprietary Information includes trade secrets, processes, formulae, data and know-how, improvements, inventions, techniques, marketing plans, strategies, forecasts and customer lists.

In consideration of my employment, or continued employment, as the case may be, and the compensation received by me from the Company from time to time, subject to Section 12 hereof, I hereby agree to the following:

1) All Proprietary Information shall be the sole property of the Company and it's assigns, and the Company and it's assigns shall be the sole owner of all patents and other rights in connection therewith. I hereby assign to the Company any rights I may have, or acquire in, all Proprietary Information. At all times during my employment by the Company, and at all times after termination of such employment, I will keep in confidence and trust all Proprietary Information, and I will not disclose, sell, use, lecture upon or publish any Proprietary Information, or anything relating to it, without the written consent of the Company except as may be necessary in the ordinary course of performing my duties as an employee of the Company.

2) During the period of my employment by the Company, I will not, without the Company's express written consent, engage, or be party to, any employment or activity which is competitive with the Company.

3) All Documents, data, records, apparatus, equipment, chemicals, molecules, organisms and other physical property, whether or not pertaining to Proprietary Information, furnished to me by the Company, or produced by myself or others in connection with my employment, shall be, and remain the sole property of the Company, and shall be returned promptly to the Company as, and when, requested by the Company. Regardless of specific request by the Company, I shall voluntarily return and deliver all such property upon termination of my employment whether initiated by me, or by the Company, for any reason and I will not remove any such property, or any reproduction of such property, from the Company's premises upon such termination.

4) For a period of not less than one year following termination of my employment with the Company, I will not solicit, or in any manner encourage, employees of the Company to leave it's employ.

5) I will promptly disclose to the Company, or any persons designated by it, all improvements, inventions, formulae, processes, techniques, know-how and data, whether or not patentable, made or conceived or reduced to practice or learned by me, either alone or jointly with others, during the period of my employment which are related to, or useful in, the business of the Company, or result from tasks assigned me by the Company, or result from use of premises owned, leased or contracted for the Company (all said improvements, inventions, formulae, processes, techniques, know-how and data shall be collectively hereinafter called 'Inventions'. Such disclosure shall continue for one year after termination of this Agreement with respect to anything that would be an Invention if made, conceived, reduced to practice or learned during the term hereof.

6) All Inventions shall be the sole property of the Company and it's assigns, and the Company and it's assigns shall be the sole owner of all patents and other rights in connection therewith. I hereby assign to the Company any rights I may have, or acquire, in all Inventions. I further agree as to all Inventions to assist the Company in every proper way, at the Company's expense, to obtain and, from time to time, enforce patents on the Inventions in any and all countries, and to that end, I will execute all documents for use in applying for, and obtaining, such patents thereon and enforcing same, as the Company may desire, together with any assignments thereof to the Company or persons designated by it. My obligation to assist the Company in obtaining and enforcing patents for the Inventions in any and all countries shall continue beyond the termination of my employment, but the Company shall compensate me at a reasonable rate after such termination for time actually spent by me at the Company's request on such assistance.

7) In the event that the Company is unable, for any reason whatsoever, to secure my signature to any lawful, and necessary, document required to apply for, or execute, any patent application with respect to an Invention (including renewals, extensions, continuations, divisions or continuations in part thereof), I hereby irrevocably designate and appoint the Company and its duly authorized officers and agents as my agents and attorneys-in-fact to act for, and in, my behalf and, instead of me, to execute and file any such application, and to do all other lawfully permitted acts to further the prosecution and issuance of patents thereon with the same legal force and effect as if executed by me.

8) As a matter of record, I have attached hereto a complete list of all inventions or improvements relevant to the subject matter of my employment by the Company which have been made or conceived, or first reduced to practice, by me alone, or jointly with others prior to my engagement by the Company, which I desire to remove from the operation of this Agreement. I covenant that such list is complete. If no such list is attached to this Agreement, I represent that I have made no such inventions and improvements at the time of signing this Agreement.

9) I represent that my performance of all the terms of this Agreement, and that my employment by the Company, does not, and will not, breech any prior agreement by me to keep in confidence proprietary information acquired by me in conjunction with any other party prior to, and continuing throughout, my employment by the Company. I have not entered into, and I agree I will not enter into, any agreement either written or oral in conflict herewith.

10) I understand, as part of the consideration for the offer of employment extended to me by the Company, and of my employment or continued employment by the Company, that I have not brought, and will not bring with me, to the Company, or use in the performance of my responsibilities at the Company, any equipment, supplies, facility or trade secret information of any former employer which are not generally available to the public, unless I have obtained written authorization for their possession and use.

11) I also understand that, in my employment with the Company, I am not to breach any obligation of confidentiality that I have to others, and I agree that I shall fulfill all such obligations during my employment with the Company.

12) In addition to any other rights and remedies available to the Company for any breach by me of my obligations hereunder, the Company shall be entitled to enforcement of my obligations hereunder by court injunction.

13) If any provision of this Agreement shall be declared invalid, illegal or unenforceable, such provision shall be severed, and all remaining provisions shall continue in full force and effect.

14) This Agreement does not apply to inventions which fully qualify for protection under Section 2870 of the California Labor Code, which are ideas or inventions for which no equipment, supplies, facility or trade secret information of the Company was used and which was developed entirely on my own time, and 1) which does not relate to (a) the business of the Company directly, or to (b) the Company's actual, or demonstrably anticipated research or development, or 2) which does not result from any work performed by me for the Company. Notwithstanding the foregoing, I shall disclose, in confidence to the Company, any invention which would permit the Company to make a determination as to compliance by me with the terms and conditions of this Agreement.

15) This Agreement shall be effective as of the first day of my employment by the Company.

16) The term Company, as used herein, shall include any subsidiary or designated affiliate of Vical, Incorporated.

17) This Agreement shall be binding upon me, my heirs, executors, assigns and administrators, and shall inure to the benefit of the Company, it's successors and assigns.

18) This Agreement shall be governed by, and construed in accordance with, the laws of the State of California.

THE FOREGOING AGREEMENT IS ACCEPTED AND AGREED TO:

Signature: _____ Date: 12/16/88

Print Name: ROBERT MALONE

AS WITNESSED BY:

Signature: _____ Date: 12/16/88

Title: Admin Mgr

ASSIGNMENT

I am the person identified below as the "Inventor." I, jointly with others, have made a certain new and useful invention (the "Invention"), as set forth in an application for United States Letters Patent, bearing substantially the following title and bearing the following serial number. The application has or will be executed by me, and it was filed on the indicated date:

Title: EXPRESSION OF EXOGENOUS POLYNUCLEOTIDE SEQUENCES IN A VERTEBRATE (CIP Application)
Serial Number: 07/467,881 Date filed: January 19, 1990

The assignee under this agreement is hereby authorized to insert the filing date and serial number referred to above, when ascertained.

For good and valuable consideration, I do hereby sell, assign, and transfer to the Wisconsin Alumni Research Foundation (hereinafter "WARF") a non-stock, non-profit Wisconsin corporation, and to its legal representatives, successors, and assigns, my entire right, title, and interest in and to the Invention and to all United States and foreign patent applications any of whose claims cover all or part of the Invention, together with all patents resulting from such applications. WARF shall hold such right, title, and interest as fully and completely as they would have been held by me had this assignment and sale not been made.

I agree, upon WARF's request, to execute or assent to such United States and foreign patent applications and to execute all separate assignments and other legal documents that WARF may find necessary or desirable in its exercise of the right, title, and interest assigned above. I also agree to communicate to WARF any facts relating to the Invention or to any of the patent applications or patents contemplated above that may be useful to WARF and to testify as to such facts in any interferences or in litigation, if requested to do so. I shall do all these things without additional compensation but at no expense to me.

I hereby request the Commissioner of Patents to issue to WARF, as the owner of my entire right, title, and interest therein, any Letters Patent of the United States that may be issued for the Invention.

Inventor: Jon A. Wolff Date: 3/15/90
Name typed or printed: Jon A. Wolff
Address: 1122 University Bay Drive
Madison, Wisconsin 53705

State of Wisconsin)
ss.)
County of Dane)

On this 13th day of March, 1990, before me personally appeared Jon A. Wolff, known to me to be the person identified above as the "Inventor," who executed the above instrument and acknowledged to me that he or she executed the same for the uses and purposes therein set forth.

SEAL

Kathleen M Eder
Notary Public

My Commission expires: 2-17-91

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